Genetic and Environmental Influences on Sleep Quality: Quantitative and Molecular Genetic Approaches to an Understanding of Individual Differences

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Declaration

I declare that the work presented in this thesis is my own. All experiments and work detailed in the text of this thesis is novel and has not been previously submitted as part of the requirements of a higher degree.

Signed _____

Date _____

Abstract

There are vast inter-individual differences in sleep quality in the general population – whilst some individuals sleep well with little or no sleep disturbance, others experience frequent sleep disturbances, problems which often manifest into chronic sleep disorders such as insomnia. The aim of this thesis is to explore factors accounting for these observed differences in sleep quality between individuals. Using data from a large-scale twin study this thesis uses behavioural genetic techniques to investigate genetic and environmental influences on sleep quality in a sample of 1,556 twins and siblings aged 18-27 years. The first four studies use quantitative genetic techniques to investigate 1) associations between components of sleep quality and the overlap in the genetic and environmental influences accounting for them; 2) specific non-shared environmental influences on global sleep quality; 3) the presence of gene-environment interplay between sleep quality and dependent negative life events; and 4) the association between sleep quality and diurnal preference, and the overlap in their aetiological influences. Most importantly, there was substantial genetic overlap between individual components of sleep quality (*rA* mostly ≥.50); sleep quality and diurnal preference ($rD = .52_{[95\%\ CI=.37-.70]}$); and sleep quality and dependent negative life events ($rD = .63_{[.45-.83]}$) – the latter finding providing evidence of gene-environment correlation. In general, non-shared environmental overlap was small (*rE* mostly ≤.40). The final study used a candidate gene approach to investigate associations between sleep quality and diurnal preference with *5HTTLPR*, *PER3*, and *CLOCK* 3111 – polymorphisms hypothesized to be implicated in sleep and/or the circadian system. An association was found between the 'long' allele of *5HTTLPR* and poor sleep quality ($\beta = -.34$, $p<.01$). This thesis utilises the twin method in novel ways in the context of sleep research and advances knowledge of the genetic and environmental underpinnings of the variation in sleep quality in healthy young adults.

Statement of Authorship

The data for this thesis come from the collaborative studies G1219 and G1219Twins (GENESiS). In these studies, I generated hypotheses for study, planned analyses and analysed data. I also assisted the fourth wave of data collection by organising and carrying out questionnaire mailouts, assisted in the mailouts of buccal swabs for the collection of DNA, and catalogued the DNA when returned. Furthermore, I re-organised the variables within the G1219 dataset from all 4 waves for the easier use of the data by new team members. In addition, I genotyped the G1219 study participants for 5 genetic polymorphisms, entered the data into the complete G1219 SPSS datafile, and wrote detailed information sheets on how to use the genotyping data. I acknowledge that the work set out in this thesis is original and my own.

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Barclay, N.L. (2011). Genetic and environmental influences on sleep quality and diurnal preference: Results from quantitative genetic and molecular genetic analyses. Paper presented at the Annual PsyPAG Conference, Bangor, Wales, 6th-8th July, 2011.

Barclay, N.L., Eley, T.C., Maughan, B., Rowe, R., Gregory, A.M. (2010). Associations between diurnal preference, sleep quality and externalising behaviours: A behavioural genetic analysis. Paper presented at the 20th Congress of the European Sleep Research Society, Lisbon, Portugal, 14th-18th September, 2010. *Journal of Sleep Research, 19 (suppl. 2), Abstract 135.*

List of Abbreviations

Δdf	Change in degrees of freedom
$\Delta\chi^2$	Change in chi-square
°C	Degrees Celsius
µl	Microlitres
-2LL	Minus twice the log-likelihood
5HT	Serotonin
5HTT	Serotonin transporter
5HTTLPR	Serotonin transporter linked promoter region
χ^2	Chi-square goodness-of-fit statistic
a	Chronbach's alpha
A	Adenine (in molecular genetics)
A	Additive genetic influence (in quantitative genetics)
a^2	Additive genetic influence
AASM	American Academy of Sleep Medicine
AIC	Akaike Information Criterion
ASPS	Advanced sleep phase syndrome
β	Standardised beta coefficient
bp	Base pair
C	Cytosine (in molecular genetics)
C	Shared (common) environmental influence (in quantitative genetics)
c^2	Shared environmental influence

CI	Confidence Interval
d	Effect size
D	Non-additive genetic (dominance) influence
d^2	Non-additive genetic (dominance) influence
df	Degrees of freedom
DLE	Dependent negative life events
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders – 4th edition
DSPS	Delayed sleep phase syndrome
DZ	Dizygotic
E	Non-shared environmental influence
e^2	Non-shared environmental influence
ECG	Electrocardiography
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
ESS	Epworth Sleepiness Scale
ESYTC	Edinburgh Study of Youth Transitions and Crime
ExE	Environment-environment interaction
G	Guanine
GWAS	Genome wide association studies
GxE	Gene-environment interaction

GxG	Gene-gene interaction
ICSD	International Classification of Sleep Disorders
ILE	Independent negative life events
kb	Kilobase
L	Long allele
MAO-A	Monoamine oxidase-A gene
MCTQ	Munich Chronotype Questionnaire
MEQ	Morningness-Eveningness Questionnaire
ml	Millilitres
mM	Micromoles
MSB	Mean-squares between twin pairs
MSW	Mean-squares within twin pairs
MZ	Monozygotic
n	Number of participants
N	Number of participants
ng	Nanograms
NREM	Non-rapid eye movement sleep
NSD	No sex differences
p	Probability
PCR	Polymerase chain reaction
PER1	Period 1 gene
PER2	Period 2 gene
PER3	Period 3 gene
PSG	Polysomnography

PSQI	Pittsburgh Sleep Quality Index
Qual. SD	Qualitative sex differences
QSD	Quantitative sex differences
QTL	Quantitative trait loci
r	Correlation coefficient
rA	Additive genetic correlation
rC	Shared environmental correlation
rD	Non-additive genetic (dominance) correlation
rE	Non-shared environmental correlation
rGE	Gene-environment correlation
REM	Rapid eye movement sleep
rpm	Revolutions per minute
S	Short allele
SAT	Saturated model fit
SCN	Suprachiasmatic nucleus
SD	Standard deviation
SDS	Sequence Detection System
SE	Standard error
SERT	Serotonin transporter
Sib	Siblings
SLC6A4	Serotonin transporter gene
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for the Social Sciences
SSD	Scalar sex differences

SSRIs	Selective serotonin reuptake inhibitors
SWS	Slow wave sleep
t	t-statistic
T	Thymine
TMS	Transcranial magnetic stimulation
UTR	Untranslated Region
UV	Ultraviolet
V_P	Total phenotypic variance
VNTR	Variable number tandem repeat

Chapter 1: Background

1.1 Overview

This chapter provides a selective review of the current literature on the epidemiology and aetiology of sleep quality in the general population and extrapolates to clinical populations where appropriate. There are 7 sections of this introductory chapter covering a range of topics directly related to the research of this thesis. First, epidemiological data are presented along with a discussion of the costs and consequences of poor sleep in the general population. Second, associations between poor sleep and psychiatric and physical conditions are outlined, and a description of the inclusion of sleep as a symptom of numerous disorders outlined in the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) (DSM-IV: American Psychiatric Association, 1994) is provided. Third, evidence for demographic differences in sleep quality relating to age and sex is presented. Fourth, objective and subjective methods for assessing sleep are described, followed by a discussion of the merits and limitations of these methods. Fifth, a review of research investigating aetiological influences on sleep quality from both quantitative genetic and molecular genetic studies is provided, as well as an examination of specific environmental factors associated with sleep quality. Furthermore, the concept of gene-environment interplay is defined and discussed in relation to psychiatric disorders in general, with a discussion of the possible involvement of gene-environment interplay in sleep. Sixth, associations between sleep quality and other aspects of normal sleep are described. Finally, areas in which there is a necessity for further research on the aetiological influences on sleep quality is described, followed by an outline of the rationale and research questions of this thesis.

1.2 Introduction

Epidemiological data have consistently shown that sleep disturbances are common in the general population (Ohayon, 2002). Indeed, there are numerous sleep disorders affecting many distinct aspects of sleep. The DSM-IV (American Psychiatric Association, 1994) describes 14 primary sleep disorders, categorised into one of two categories: parasomnias – which are characterised by atypical behaviours which occur during the sleep period, for example sleepwalking; and dyssomnias – which are characterised by abnormalities in the timing, duration or quality of sleep, including problems such as insomnia (and its subtypes) and circadian rhythm sleep disorders. An alternative classification system, the International Classification of Sleep Disorders (ICSD-2: American Academy of Sleep Medicine, 2005), provides an even more comprehensive list of sleep disorders present in the general population.

In addition to clinically diagnosed sleep disorders, there is wide variation in *normal* sleep characteristics in the population. Whilst some individuals sleep soundly with little or no disturbance from sleep, others may experience poor sleep quality and have frequent disturbances. Poor sleep quality (and at the extreme, problems such as insomnia) is typically characterised by difficulty initiating or maintaining sleep, early morning awakenings, feeling that the sleep period is non-restorative or unrefreshing, and is associated with significant impairments in daytime functioning (Buysse, Ancoli-Israel, Edinger, Lichstein, & Morin, 2006; Ohayon, 2002; Roth, 2007). Numerous studies assessing sleep disturbances across the USA, Canada, and Western Europe including the UK have repeatedly reported that approximately one third of the general population frequently experience at least some insomnia symptoms (for example, see Coren, 1994; Karacan, et al., 1976; Leger, Guilleminault, Dreyfus, Delahaye, & Paillard, 2000; Morin, LeBlanc, Daley, Gregoire, & Merette, 2006; Morphy, Dunn, Lewis, Boardman, & Croft, 2007; Ohayon, Caulet, Priest, & Guilleminault, 1997;

Ohayon & Reynolds, 2009; Ohayon, Roberts, Zulley, Smirne, & Priest, 2000; Weyerer & Dilling, 1991), and that around 6-10% meet diagnostic criteria for insomnia according to the DSM-IV (Ford & Kamerow, 1989; Leger, et al., 2000; Morin, et al., 2006; Ohayon, Caulet, & Guilleminault, 1997; Ohayon & Reynolds, 2009). Indeed, insomnia is considered to be one of the most common health problems in the general population (Hublin & Partinen, 2002).

Given that sleep disturbances are so prevalent in the general population, it is not surprising that poor sleep poses a significant public health problem, in terms of financial costs and increased use of health care services (Espie, 2002; Leger & Bayon, 2010). A 1995 survey in the United States estimated that the direct cost of insomnia totalled approximately $14 billion per year (Walsh & Engelhardt, 1999). Other studies have also indicated the direct costs of sleep disorders worldwide to be substantial (Daley, Morin, LeBlanc, Gregoire, & Savard, 2009). These costs are largely due to more frequent consultations with healthcare providers, medical examinations and use of medications in individuals experiencing sleep disturbances (Daley, Morin, LeBlanc, Gregoire, & Savard, 2009; Leger, Guilleminault, Bader, Levy, & Paillard, 2002). In addition to the substantial direct costs involved in treating sleep disturbances, poor sleep is also associated with significant indirect costs, such as a greater risk of accidents (Daley, Morin, LeBlanc, Gregoire, Savard, et al., 2009) including motor vehicle accidents (Ohayon & Smirne, 2002; Pizza, et al., 2010), and impaired daytime functioning including reduced concentration, impaired work performance and productivity, and work absenteeism (Daley, Morin, LeBlanc, Gregoire, & Savard, 2009; Godet-Cayre, et al., 2006; Leger, et al., 2002; Linton & Bryngelsson, 2000; Rosekind, et al., 2010).

1.3 Comorbidity

Poor sleep quality is known to be associated with many behavioural, physical and emotional phenotypes. For example, sleep disturbances are more prevalent in individuals with psychiatric or chronic physical disorders than in the general population (Ancoli-Israel, 2006). Large scale community samples have indicated that sleep disturbances are most commonly comorbid with psychiatric disorders such as anxiety (including generalised anxiety disorder, phobias, obsessive-compulsive disorder and panic disorder), and depression (Ford & Kamerow, 1989; Morin & Ware, 1996; Weyerer & Dilling, 1991). In addition sleep disturbances have been found to be comorbid with a range of other psychiatric and psychological disorders such as alcohol and drug abuse (Ford & Kamerow, 1989), personality and psychotic disorders (Nowell, et al., 1997), schizophrenia, and somatisation disorder (Weissman, Greenwald, Nino-Murcia, & Dement, 1997). As well as being comorbid with other disorders, several longitudinal studies have suggested that sleep disturbances may be a risk factor for the development of chronic psychiatric disorders, such as depression and anxiety (Breslau, Roth, Rosenthal, & Andreski, 1996; Ford & Kamerow, 1989; Gregory, Caspi, et al., 2005; Gregory, Rijsdijk, Lau, Dahl, & Eley, 2009; Johnson, Roth, & Breslau, 2006; Livingston, Blizard, & Mann, 1993; Neckelmann, Mykletun, & Dahl, 2007), panic disorder (Weissman, et al., 1997) and alcohol and drug use in adults (Wong, Brower, Fitzgerald, & Zucker, 2004; Wong, Brower, Nigg, & Zucker, 2010), as well as aggression and attention problems in children (Gregory & O'Connor, 2002). In a US study which assessed the presence of sleep and psychiatric complaints at two time points, individuals with insomnia at baseline were 40 times more likely to develop a new major depression and 6 times more likely to develop an anxiety disorder at follow-up compared to those without insomnia (Ford & Kamerow, 1989). In a study in the UK, the presence of insomnia at baseline was associated with approximately 3 times greater

risk of developing depression 12 months later and twice the risk of developing anxiety disorder (Morphy, et al., 2007).

However, understanding of the causal mechanisms between sleep and psychopathology is complicated by the fact that associations are likely to be bidirectional (Franzen & Buysse, 2008; Kim, et al., 2009). For example, substantial evidence suggests that depressive symptoms are an important risk factor for the development and persistence of sleep problems (Patten, Choi, Gillin, & Pierce, 2000; Quan, et al., 2005). Another factor complicating the understanding of the causal mechanism between sleep and psychopathology is that sleep disturbances are often symptoms of, rather than comorbid with, other difficulties. Indeed, the DSM-IV describes no less than 19 psychological disorders where difficulties with sleep are listed as a symptom or where problems with sleep are a consequence of another underlying condition (American Psychiatric Association, 1994), and the ICSD-2 describes over 30 disorders where insomnia symptoms are also present (American Academy of Sleep Medicine, 2005). The question of whether sleep disturbances, in particular insomnia symptoms, are considered to warrant a clinical diagnosis in their own right, or whether they are simply symptoms of other disorders, has received a great deal of attention in the literature (for example, see Harvey, 2001).

As well as psychiatric disorders, poor sleep is associated with physical health, well-being and mortality. For example, sleep disturbances are associated with physical conditions such as hypertension, gastro-esophageal reflux, heart disease, hypercholesterolemia, chronic pain and obesity amongst others (Algul, et al., 2009; Ohayon, 2009b; Vgontzas, et al., 1998) (although it should be noted that there is controversy within the field as to associations between sleep and obesity, see Horne, 2008); and sleep disturbances are often common symptoms of neurological diseases such as Alzheimer's, Parkinson's and Huntington's (Harvey, 2001). In terms of well-

being, sleep satisfaction is associated with greater well-being (Jean-Louis, Kripke, & Ancoli-Israel, 2000), and good sleep quality is associated with life satisfaction (Pilcher, Ginter, & Sadowsky, 1997), which may be a presage to good health-related quality of life in late adulthood (Driscoll, et al., 2008). Furthermore, poor quantity as well as quality of sleep has been shown to have deleterious effects on health. For example, short sleep duration is associated with coronary heart disease (Chandola, Ferrie, Perski, Akbaraly, & Marmot, 2010), self perceptions of poor general health (Steptoe, Peacey, & Wardle, 2006), and two recent meta-analyses have concluded that both long and short sleep duration are significant risk factors for type 2 diabetes (Cappuccio, D'Elia, Strazzullo, & Miller, 2010a) and all-cause mortality (Cappuccio, D'Elia, Strazzullo, & Miller, 2010b).

These findings, together with data outlining the economic cost of sleep disturbances, highlight the need for a more comprehensive understanding of the causes of variation in sleep disturbance observed in the general population. Investigation of the genetic and environmental underpinnings of sleep disturbance may have implications for the development of treatments or identification of risk factors that may lead to its occurrence.

1.4 Demographics

1.4.1 Age

Previous research has consistently demonstrated that sleep-wake patterns become more fragmented with age (Bliwise, 1993; Carskadon, Brown, & Dement, 1982; Miles & Dement, 1980; Park, Matsumoto, Seo, Kang, & Nagashima, 2002). Specifically, objective data from laboratory studies have identified changes in sleep architecture that occur in older as compared to younger adults, such as lower percentage

of slow wave sleep (SWS – stages 3 and 4 of non-REM sleep) and rapid eye movement sleep (REM), and a higher percentage of stage 1 and 2 sleep (Buysse, et al., 1991) (for more information on the stages of sleep see section 1.5.1). This pattern has also emerged when assessing age linearly across the life-course (between ages 20-59 years) (Carrier, Monk, Buysse, & Kupfer, 1997) - findings which have more recently been confirmed by a meta-analysis of data from 65 studies from 1960-2003 (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). Subjective reports also indicate that, compared to younger adults, older adults typically experience poorer sleep quality (Buysse, et al., 1991; Hoch, et al., 1997), less time asleep, decreased sleep efficiency, more awakenings from sleep (Carrier, et al., 1997), difficulty sleeping (Karacan, et al., 1976) and an increased incidence of insomnia (Ford & Kamerow, 1989). Results from a French community sample showed a linear association between increasing age and higher percentage of individuals reporting mild as well as moderate/severe insomnia symptoms in several age cohorts ranging from adolescence to late adulthood (Weyerer & Dilling, 1991). This finding has also been confirmed by a similar study in the UK (Ohayon, 1997), a study of older adults in Hong Kong (Chiu, et al., 1999), and in a Turkish sample age was linearly associated with poor sleep quality (Atalay, 2011). In addition research suggests that the association between sleep duration and mortality is stronger in older as compared to younger adults (Kripke, Simons, Garfinkiel, & Hammond, 1979).

Contrary to this, other studies suggest that the association between sleep disturbance and age is less clear-cut. For example epidemiological data from Leger and colleagues (2000) showed that insomnia symptoms increased with age in those aged between 25-34 years, but that this association disappeared after 35 years of age. Furthermore, in children, it has been found that sleep problems *decreased* from childhood to adolescence (Gregory & O'Connor, 2002). Other reports have failed to find

significant associations with age and subjective sleep quality (Carrier, et al., 1997; Middelkoop, Smilde-van den Doel, Neven, Kamphuisen, & Springer, 1996), and insomnia symptoms (Liljenberg, Almqvist, Hetta, Roos, & Agren, 1988; Sutton, Moldofsky, & Badley, 2001).

1.4.2 Sex

It is a commonly held view that sleep complaints are more prevalent in females than males, and indeed numerous studies report evidence to support this claim (for example, see Ford & Kamerow, 1989; Groeger, Zilstra, & Dijk, 2004; Karacan, et al., 1976; Leger, et al., 2000; Ohayon, 2002; Weyerer & Dilling, 1991). This finding has been confirmed in a meta-analysis of 31 studies of insomnia or insomnia type symptoms, demonstrating a risk ratio of 1.41 of females experiencing greater symptoms compared to males (Zhang & Wing, 2006). Likewise, in a review of 33 studies assessing subjective insomnia (including problems such as difficulty initiating sleep, difficulty maintaining sleep, and early morning awakening), Lichstein and colleagues (2004) noted that 73% of studies reported a greater prevalence of insomnia complaints in women (Lichstein, Durrence, Riedel, Taylor, & Bush, 2004). Despite strong evidence for a sex difference in insomnia symptoms, studies that have assessed subjectively defined *sleep quality* are somewhat inconsistent. Some studies have found evidence in accordance with this finding (Lindberg, et al., 1997; Middelkoop, et al., 1996; van den Berg, et al., 2009; Vitiello, Larsen, & Moe, 2004), whilst others have found no evidence for significant sex differences in subjectively defined sleep quality (Atalay, 2011; Buysse, Hall, et al., 2008; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989; Buysse, et al., 1991; Carpenter & Andrykowski, 1998; Sakakibara, et al., 1998). Such inconsistency in research investigating sex differences in sleep is also reflected in studies using objective measures. For example, one study found that women showed

better sleep than men as indicated by several indices of polysomnographically defined sleep (such as shorter sleep onset latency, higher sleep efficiency, longer sleep duration) (Goel, Kim, & Lao, 2005) (see section 1.5.1 for more information on polysomnography). Contrastingly, Buysse and colleagues (2008) demonstrated that healthy good sleeping women showed higher power in the theta band compared to men, and that women with primary insomnia exhibited higher delta, theta, sigma, beta and gamma power compared to men with primary insomnia. It is possible that such increased high and low frequency EEG activity reflects a state of hyperarousal, and consequently, disturbed sleep. However, Voderholzer and colleagues (2003) found no such differences between males and females in polysomnographically defined sleep measures (including sleep duration, sleep efficiency, arousal index, percentage of time spent awake, and percentage of time in slow wave sleep and REM sleep). Using actigraphy to assess sleep (see section 1.5.1 for more information on actigraphy), van den Berg and colleagues (2009) demonstrated that women experienced longer sleep duration and less fragmented sleep than men, despite subjective reports from the same participants demonstrating poorer sleep quality in women. These inconsistencies suggest that the differences in sleep of men and women are unclear and that other study specific characteristics may be accounting for the observed differences as outlined above (for example, age).

1.5 Measuring Sleep

1.5.1 Polysomnography and actigraphy

A common method to objectively assess sleep is polysomnography (PSG). PSG refers to the collection of physiological variables during sleep. Typically PSG measures the neural electroencephalograph (EEG) to assess brain activity, electrooculography

(EOG) to measure eye movements, electromyography (EMG) to measure muscle activity, and electrocardiography (ECG) to assess heart rhythms. Using PSG, Rechtschaffen and Kales (1968) identified and documented 5 distinct stages of sleep that exhibit highly specific EEG characteristics. Stage 1 is the beginning of sleep and is characterised by high amplitude theta waves (~4-7 Hz) which are slower in frequency than alpha waves (~8-13 Hz) (typically observed during relaxed wakefulness). Stage 1 usually only lasts around 1-7 minutes before the transition to stage 2. Stage 2 is characterised by sleep spindles – a sudden increase in wave frequency (~12-14 Hz); and k-complexes – a sudden increase in wave amplitude. These phenomena occur periodically during stage 2 along with a general decrease in muscle tone. Stages 3 and 4 are characterised by delta waves (~0-4 Hz) and are often termed 'deep sleep' or 'slow-wave sleep' (SWS) (as opposed to the 'light sleep' of stages 1 and 2). Stages 3 and 4 are differentiated by the percentage of delta waves present – if less than 50% of the waves are delta waves, sleep is considered to be stage 3; whereas if more than 50% of the waves are delta waves, sleep is considered to be stage 4 (Rechtschaffen & Kales, 1968). However, more recently refined methods for scoring sleep combine stages 3 and 4, and consider stage 3 to be characterised simply by greater than 20% delta wave activity (AASM: American Academy of Sleep Medicine, 2007). The first 4 stages (or first 3 stages using AASM criteria) can be described as non-REM (NREM) sleep. The final stage of sleep, rapid eye movement (REM) sleep, however, is entirely distinct and is characterised by EEG patterns analogous to those of the waking state, including alpha and beta waves, and is accompanied by rapid darting eye movements and a dramatic loss of muscle tone. During the night, an individual will typically move down through the sleep stages periodically, with one cycle lasting ~90 minutes. The cycle is repeated throughout the night with the exception that stage 1 is replaced with REM, with each REM period becoming successively longer throughout the night.

This method of measuring sleep provides information about depth of sleep by assessing the transition through the 4 sleep stages (when scored using the AASM method; 5 stages when using the method proposed by Rechtschaffen & Kales, 1968), as well as information regarding sleep disturbances and movements. From these measurements sleep quality can be quantified by the observed fragmentation, consolidation, or duration of sleep stage episodes (Franken & Tafti, 2003). Although PSG is typically performed in a laboratory, the development of portable PSG devices has meant that these measurements can also be assessed in the home environment (see Broughton, Fleming, & Fleetham, 1996 for a review).

Another objective method of measuring sleep is actigraphy. Actigraphy involves participants wearing a watch-like device, an actigraph, when they go to bed and throughout the day. This device monitors activity and movements in one minute epochs during the night to give an indication of sleep duration and arousals (see Sadeh, 2011, for a review of the role of actigraphy in sleep medicine).

1.5.2 Sleep diaries and questionnaires

Although PSG is often regarded as the "gold standard" method to assess sleep, subjective reports of sleep quality are widely used in research, especially in large scale studies when objective measures may be too costly and time consuming to carry out. Sleep diaries are reliable at providing information regarding sleep-wake patterns over a 24-hour period (Rogers, Caruso, & Aldrich, 1993), and require the participant to describe events preceding the sleep period as well as to describe the sleep period after awakening (Monk, et al., 1994). In addition to sleep diaries, questionnaire measures of sleep are also widely used in research. The advantage of questionnaires is that they are simple and quick to complete, and provide an accurate insight into many aspects of sleep over a given time period – making them the most feasible method of obtaining

data in very large studies. There are several questionnaire measures that are commonly used in sleep research and medicine. For example, daytime sleepiness is commonly assessed by the Epworth Sleepiness Scale (ESS: Johns, 1991) and the Sleep-Wake Activity Inventory (Rosenthal, Roehrs, & Roth, 1993); and insomnia symptoms are often assessed by the Insomnia Severity Index (Bastien, Vallieres, & Morin, 2001; Morin, 1993), and have recently begun to be assessed by the Insomnia Symptoms Questionnaire (Okun, et al., 2009). An alternative method of assessing insomnia symptoms is to ask questions based on the DSM-IV diagnostic criteria for primary insomnia. In such instances, individuals are asked, for example, how frequently they (i) have difficulty initiating or maintaining sleep, (ii) experience non-restorative sleep, and (iii) suffer daytime consequences of poor sleep (see Ohayon, 2002 for a discussion of the assessment of insomnia symptoms). However, simply measuring the frequency of insomnia symptoms may not necessarily give an indication of the perceived subjective quality of one's sleep. Ohayon and colleagues (1997) have shown that although around a third of the population experience some insomnia symptoms, not all of these individuals are dissatisfied with their sleep. Indeed, the proposed revisions to the DSM (scheduled for publication in 2013, American Psychiatric Association, 2010) have included as the primary symptom of 'Insomnia Disorder' dissatisfaction with sleep quantity or quality. This suggests that perhaps an alternative way to determine sleep problems is not to ask about insomnia symptoms such as sleep onset or maintenance problems *per se*, but to more directly assess indices of subjective satisfaction and quality of sleep. The most widely used self-report measure to assess subjective sleep quality to date is the Pittsburgh Sleep Quality Index (PSQI: Buysse, et al., 1989). The PSQI assesses overall sleep quality and disturbances in the past month and taps into seven distinct components of sleep quality: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and

daytime dysfunction. This measure has been used in numerous clinical and non-clinical settings, has excellent diagnostic sensitivity in distinguishing good from poor sleepers, and is considered one of the best measures for assessing indices of global sleep and insomnia symptoms (Buysse, et al., 2006). For further discussion of the psychometric properties of the PSQI, see Chapter 3.

In addition to assessing specific sleep disturbances and indices of sleep quality, there are a number of questionnaires which assess indices of sleep timing and circadian rhythmicity. For example, the Sleep Timing Questionnaire (Monk, Buysse, et al., 2003) was specifically designed to capture information on sleep timing that would otherwise be collected using sleep diaries over a two week period. An alternative measure for assessing the timing of sleep is the Munich Chronotype Questionnaire (MCTQ: Roenneberg, Wirz-Justice, & Merrow, 2003), which considers the actual timing of sleep on work days and free days and number of hours of light exposure. Perhaps a more common questionnaire for assessing the *preferred* timing of sleep-wake schedules, however, is the Morningness-Eveningness Questionnaire (MEQ: Horne & Östberg, 1976). The MEQ assesses individuals' preference for daytime and nighttime activities and hours of peak performance to determine one's diurnal preference – an indicator of circadian rhythmicity (diurnal preference will be discussed at length in Chapter 6). The MEQ has been found to correlate well with the MCTQ, particularly with the timing of sleep on free compared to work days (Zavada, Gordijn, Beersma, Daan, & Roenneberg, 2005).

1.5.3 Objective vs. subjective measures

The decision of which methods to use to assess sleep largely depends on the specific research project proposed. Polysomnographic data would be essential, for example, in studies investigating sleep architecture, structure and sleep stage transitions,

however the collection of such data is not always necessary (for example in studies assessing subjective sleep quality), and in large scale studies as in the present research inclusion of polysomnography is too costly and time-consuming. There are advantages and disadvantages to using objective and subjective measures to assess sleep. Objective measures such as PSG record an accurate, unbiased description of the entire sleep period, provide detailed information on neural activity, and have the ability to differentiate sleep disturbances and uncover masked sleep disorders. However, PSG is usually carried out in a sleep laboratory and is an invasive procedure in which participants are connected to many electrodes throughout the night. Although many experimental paradigms typically carry out an "adaptation night", in which the participant is introduced to the laboratory and spends one night there prior to the experiment, sleep in the laboratory may not be an accurate description of one's usual sleep-wake patterns in the home environment (Edinger, et al., 1997). Indeed evidence suggests that sleep quality and efficiency are significantly better in the home environment compared to the laboratory setting (Kingshott & Douglas, 2000). However, the use of portable home-based PSG devices has overcome this issue. Nonetheless, home-based PSG has a number of caveats, including the fact that the number of EEG channels used is restricted, the patient may have technical difficulties with the equipment, and the home conditions are difficult to control (Broughton, et al., 1996). The recent development of more ecologically valid laboratory settings, however, will greatly improve future sleep research assessing sleep in an almost perfect laboratory environment.

With regards actigraphy, despite many advantages to using this method, including its validity and reliability and the non-invasive nature of the procedure, one disadvantage is that it has a low ability of detecting periods of motionless wakefulness which may occur in individuals experiencing difficulties with sleep onset, such as in

insomnia (Sadeh, 2011). This is because actigraphy is often limited to measuring movement only. In addition to these caveats, objective methods such as PSG or actigraphy provide no information as to the potential daytime consequences of a poor night's sleep - a feature which is included in the diagnostic criteria for certain sleep disorders (e.g. insomnia, American Psychiatric Association, 1994). Furthermore, a paradoxical finding is that some individuals perceive their sleep as poor and experience daytime consequences consistent with the presence of a sleep disturbance even in the absence of objective laboratory based evidence of a problem (often termed 'sleep-state misperception') (Edinger & Krystal, 2003; Morin, 2000; Trinder, 1988). Therefore, PSG alone would not identify individuals with subjective sleep complaints. With this in mind it is not surprising that studies comparing laboratory based methods and self-reports to assess sleep have consistently shown that subjective sleep complaints do not always correlate with polysomnographically defined problems (see Gregory, Cousins, et al., 2011 for an example of this phenomenon in adolescents). Generally, individuals with insomnia significantly overestimate their sleep onset latency and underestimate the quantity and quality of their sleep compared to objective data (Carskadon, et al., 1976; Edinger & Fins, 1995; Manconi, et al., 2010) – a pattern which has also been found in healthy adults (Baker, Maloney, & Driver, 1999). In contrast to this general finding, one study assessing subjective sleep quality by the PSQI found that subjectively defined sleep quality correlated well with objective sleep measures in young adults (aged between 20-30 years), but did not in older adults (aged 80+ years) (Buysse, et al., 1991). Whilst the possible inaccuracy of individuals' self reports of sleep are a disadvantage of the use of subjective measures, these findings suggest that assessing an individual's subjective experience or perception of sleep is an informative method of distinguishing good from poor sleepers. However, assessing sleep using multiple

measures, both subjective and objective, would be optimum although not always feasible.

1.6 The Aetiology of Sleep-Wake Behaviour and Causes of Variation in Sleep in the General Population

The regulation of sleep-wake behaviour, including the timing, duration and quality of sleep, is generally considered to be the product of two processes: a *homeostatic* and a *circadian* process (Borbely, 1982, 1998; Daan, Beersma, & Borbely, 1984). In the homeostatic process sleep propensity, or 'Process S', rises during the day and decreases during sleep. Sleep propensity is dependent on sleep need, indexed by prior sleep and waking. The circadian process, however, maintains 24-hour daily rhythms by a self-sustained oscillator located in the suprachiasmatic nuclei of the hypothalamus (SCN) which ensures entrainment of biological rhythms to external time-cues, thus influencing the timing of sleep, independent of the homeostatic process. These processes are considered to develop independently and it is likely that many genetic and environmental factors are involved (Gregory & Franken, 2009). The trait-like stability of sleep within individuals and the vast variability of 'normal' sleep patterns between individuals in the general population further suggests that the variation in sleep phenotypes between individuals may be explained by underlying genetic and environmental factors.

There are several diverse methods to investigate the aetiology of a trait. Animal studies using mice or drosophila provide a way of determining a specific gene's function. By inducing mutations in genes (mutagenesis), or breeding strains of 'knock-out' mice in which a certain gene is entirely abolished, researchers can examine phenotypic variations that occur due to the genetic manipulation. As an example of

research using such methods, Wisor and colleagues (2003) demonstrated a role for the serotonin transporter (SERT) gene in REM sleep regulation by observing higher rates of REM in SERT knock-out mice compared to wild types. This type of research is a 'reverse genetic' approach in that it assesses associations from genotype to phenotype. Although research of this kind gives a direct indication of the possible universal function of the genes manipulated, ethical considerations prevent such studies from being performed in humans, and so investigation of the role of genetics in sleep is accomplished by using natural experiments. Such experiments can be described as 'forward genetic' approaches in that they test associations from phenotype to genotype and assess the possible role of genetic influences in explaining individual differences in behaviour (see Gregory & Franken, 2009, for a review of genetic approaches used in sleep research). In one such forward genetic design, Wu and colleagues (2008) examined sleep in drosophila in order to identify mutant genes for short sleep. Wu et al. observed that short sleeping flies tended to sleep in short bouts, suggesting that they experienced symptoms analogous to sleep maintenance insomnia, and that this phenotype mapped onto a novel allele of the dopamine transporter gene.

In humans, a useful method to investigate the aetiology of any given trait (phenotype) of interest is to use genetically sensitive designs. The occurrence of different types of twins (identical/monozygotic [MZ]; and non-identical/dizygotic [DZ]) provide the perfect natural experiment and twin studies are one such method for investigating the extent to which genetic and environmental influences are important for a phenotype or co-occurring phenotypes. Whilst twin studies do not identify specific genes associated with traits, knowledge of the possible contribution of genetic influences on a trait or co-occurring traits may guide molecular genetic research. Although it is beyond the scope of this chapter to discuss these techniques in more detail, a brief overview of these techniques and details of key studies from quantitative

and molecular genetic studies of sleep are outlined below. Further discussion of both quantitative and molecular genetic techniques is provided in Chapter 2.

1.6.1 Quantitative genetics

The twin method relies on the genetic relatedness of monozygotic (MZ) twins who share 100% of their genetic make-up, and dizygotic (DZ) twins who share on average only half of their segregating genes (the same genetic relatedness as full siblings) to make assumptions about the aetiology of a trait. By comparing the similarity within pairs of MZ twins to the similarity within pairs of DZ twins and full siblings on a particular trait of interest (for example scores on a measure of sleep quality measured separately between the twins), we can estimate the relative contribution of additive genetic, non-additive genetic (dominance), shared environmental and non-shared environmental influences on traits using model fitting and regression based analyses (see Plomin, DeFries, McClearn, & McGuffin, 2008). Additive genetic influences can be described as the cumulative effect, or the 'adding up', of genes to influence behaviour. Non-additive genetic influences can be described as the interaction of genes at a locus. Shared environmental influences are those that are shared between twins within a family which act to make family members similar (such as socioeconomic status or living near a busy road). Non-shared environmental influences can be described as environmental influences unique to each twin/sibling within a family which account for their dissimilarity (such as one twin experiencing an accident or being involved with deviant peers). Greater similarity within pairs of MZ twins compared to DZ twins indicates that genetic influences may be important for a trait. Similar MZ/DZ correlations indicate the importance of the shared environment. MZ twin correlations less than one indicate a role for the non-shared environment (for further discussion of how these sources of variance are calculated, see Chapter 2).

Twin studies have consistently demonstrated that genetic influences are important for a variety of sleep phenotypes. Using EEG to assess the sleep of twins, several lines of evidence have shown that sleep stages 2 and 4 and delta wave sleep have a strong genetic component, whilst REM sleep appears to be largely determined by non-genetic factors (Ambrosius, et al., 2008; Linkowski, Kerkhofs, Hauspie, & Mendlewicz, 1991; Linkowski, Kerkhofs, Hauspie, Susanne, & Mendlewicz, 1989). Furthermore, genetic factors have been implicated in the 24-hour cortisol profile – a strong indicator of circadian rhythmicity (Linkowski, et al., 1993). Data from subjective reports of sleep from twins have demonstrated that around 30-50% of the variance in subjective sleep quality is accounted for by genetic influence (Heath, Kendler, Eaves, & Martin, 1990; Partinen, Kaprio, Koskenvuo, Putoken, & Langinvainio, 1983). Similar heritability estimates have been found for subjectively defined sleep disturbances, sleep onset latency, sleep length, sleep time, daytime napping and sleepiness, parasomnias and insomnia symptoms in non-clinical adult populations (Carmelli, Bliwise, Swan, & Reed, 2001; Heath, Eaves, Kirk, & Martin, 1998; Heath, et al., 1990; Hublin, Kaprio, Partinen, & Koskenvuo, 2001; McCarren, Goldberg, Ramakrishnan, & Fabsitz, 1994; Partinen, et al., 1983; Watson, Goldberg, Arguelles, & Buchwald, 2006), and in school-age children (Gregory, 2008; Gregory, Rijsdijk, & Eley, 2006); as well as indices of circadian rhythm phase such as diurnal preference (Hur, Bouchard, & Lykken, 1998; Koskenvuo, Hublin, Partinen, Heikkila, & Kaprio, 2007; Vink, Groot, Kerkhof, & Boomsma, 2001) (see Chapter 6 for a detailed discussion of diurnal preference). In these studies sleep was assessed by questionnaires in which questions about sleep usually referred to nights during the past weeks or months, demonstrating the heritability of somewhat stable sleep characteristics. However, one study which assessed several aspects of subjectively defined sleep by use of a one night sleep diary only, showed that there was no familial component to subjective sleep quality

(Boomsma, van Someren, Beem, de Geus, & Willemsen, 2008). It is possible that this discrepancy between subjective reports of sleep referring to the past weeks or months and those referring to the previous night only reflects the night-to-night variability of sleep. Indeed, it has been shown that individuals with insomnia often experience variable sleep patterns, where several nights of poor sleep may be followed by a night of relatively good sleep (Vallières, Ivers, Bastien, Beaulieu-Bonneau, & Morin, 2005; Vallières, Ivers, Beaulieu-Bonneau, & Morin, 2011). Thus, results referring to the previous night only may not capture the true picture of sleep quality due to such night-to-night variability.

1.6.2 Molecular genetics

Whilst twin studies allow us to determine the relative importance of genetic influences compared to non-genetic factors, molecular genetic research enables us to identify specific gene variants (polymorphisms) implicated in sleep. There are two main methods used in molecular genetics to identify genes associated with any given phenotype under study in humans: linkage and association. Typically, linkage refers to the process whereby a particular disease/trait is traced back through generations within large family pedigrees alongside a particular segment of DNA (deoxyribonucleic acid) which is more common in the presence of the disease/trait under study (Plomin, et al., 2008). A more common linkage approach, however, uses affected sibling pairs rather than many generations of large families. The affected sib-pair linkage design is based on allele sharing between siblings, i.e. whether affected siblings share 0, 1 or 2 alleles from their parents. Using this information it is possible to calculate the probability that a marker is linked to a gene which influences a particular disorder or quantitative trait in several pairs of affected siblings (Plomin, et al., 2008). Whilst this technique is useful in identifying genes associated with monogenic traits (i.e. diseases/traits that are caused

entirely by the effects of one gene, for example Huntington disease), the majority of complex traits showing wide variation within the population are largely influenced by many genes each having a small effect – QTLs (quantitative trait loci) (Plomin, et al., 2008). Linkage studies are significantly underpowered to detect modest gene effects, especially if the marker allele under study is relatively rare in the population and if several genes are necessary for the trait (Risch & Merikangas, 1996). Association studies, however, have greater power to detect even genes of small effect. Rather than focussing on the co-transmission of a particular marker allele and a disease/trait as in linkage, association studies compare the frequencies of gene variants in those with and without the disease/trait under study (cases and controls), or in the case of quantitative traits, high versus low scorers on a particular measure (Plomin, et al., 2008). The disadvantage of association studies, however, is that the gene or genes to be studied must be tentatively identified as possible candidates for association prior to investigation (Risch & Merikangas, 1996).

In cases where there are no *a priori* assumptions as to which genes may be involved in a phenotype, an increasingly popular method for mapping genotype to phenotype involves genome wide association studies (GWAS). Using microarrays (small chips that can hold large amounts of DNA), GWAS can genotype the entire genome for hundreds of thousands of single nucleotide polymorphisms (SNPs) simultaneously (Plomin, et al., 2008). This method may uncover physiological systems involved in a phenotype that were previously unknown (Franken & Tafti, 2003). However, where candidate genes are known, genotyping single genes may be more appropriate and cost-effective.

The search for candidate genes influencing sleep has largely focussed on the role of serotonin (5-hydroxytryptamine, 5HT) in sleep quality and insomnia and the role of clock genes in relation to circadian phenotypes. Serotonin function is a fundamental part

of the body's homeostatic system, driving sleep/wake behaviour (Jouvet, 1972), and influences a broad range of physiological functions related to appetite, aggression, cognition, endocrine regulation, mood, motor function, sexual behaviour, and pain sensitivity (Heninger, 1997; Lucki, 1998). Increased serotonin activity in the dorsal raphe nucleus of the brain stem is associated with wakefulness, and it is thought that the build up of sleep propensity during the day is partly attributed to these neurons (Adrien, 1995). Furthermore, 5HT has been associated with sleep stage characteristics. Specifically, reducing $5HT_2$ receptor activity increases slow wave sleep (SWS) and slow wave activity (SWA) - implicating these receptors in SWS regulation (Landolt, et al., 1999). Additionally, administration of selective serotonin reuptake inhibitors (SSRIs) has been shown to increase stages 1 and 2 sleep, and decrease SWS and REM sleep, and is associated with poorer sleep quality assessed objectively by PSG (Oberndorfer, Saletu-Zyhlarz, & Saletu, 2000). Recently, attention has focussed on the transporter region of the 5HT gene – *5HTTLPR* (serotonin transporter linked promoter region). A common 44 base pair (bp) deletion of *5HTTLPR*, which constitutes a 'short' allele, has repeatedly been associated with anxiety and depression symptoms (for example, see Collier, et al., 1996; Lesch, et al., 1996). Although few studies to date have investigated the role of *5HTTLPR* in sleep, those to date have found associations between the 'short' allele and primary insomnia (Deuschle, et al., 2010), and poor sleep quality in individuals experiencing chronic stress (Brummett, Krystal, Ashley-Koch, et al., 2007). Since the 5HT transporter gene is important for controlling serotonin function, polymorphisms of this region may result in sleep disturbances by inhibiting the deactivation of serotonin neurons at sleep onset. However, further research is required to confirm the role of this polymorphism in sleep quality in the general population. The role of *5HTTLPR* in relation to sleep will be discussed in greater detail in Chapter 7.

In addition to investigating serotonin, molecular genetic research has focussed on a group of '*CLOCK*' genes in relation to sleep and circadian phenotypes which includes *CLOCK, BMAL1,* three *Period (PER)* genes and two *Chryptochrome (CRY)* genes. These '*CLOCK*' genes are involved in a feedback loop which controls the circadian system (Reppert & Weaver, 2001). The *CLOCK* and *BMAL1* genes are positive transcriptional regulators which drive the expression of the negative elements of the loop, *PER1, 2* and *3*, as well as *CRY 1* and *2* via their protein products, CLOCK and BMAL1 (Wulff, Porcheret, Cussans, & Foster, 2009). Polymorphisms of these genes have been associated with numerous sleep phenotypes and disorders. For example, variants of *CLOCK* have been found to be associated with sleep duration in two independent general population samples (Allebrandt, et al., 2010). Furthermore, a SNP in the 3' untranslated region (UTR) of *CLOCK* at position 3111 consisting of a 'C' to 'T' substitution, has been identified whereby the 'C' allele is associated with shorter sleep duration and delayed sleep onset in bipolar depressed patients (Benedetti, et al., 2007), reoccurrence of insomnia in depressed patients (Serretti, et al., 2003), and a preference for eveningness in the general population (Katzenberg, et al., 1998). The *PER* genes have also been associated with disorders of sleep timing. For example, SNPs in both *PER1* and *PER2* are associated with advanced sleep phase syndrome (ASPS) – a disorder characterised by an extreme shift in circadian rhythmicity such that sleep time is advanced and wake time occurs in the early hours of the morning (Carpen, von Schantz, Smits, Skene, & Archer, 2006; Toh, et al., 2001). Similarly, a length polymorphism in *PER3* has been associated with delayed sleep phase syndrome (DSPS) whereby sleep timing is severely delayed, and extreme diurnal preference – i.e. extreme 'morningness' or 'eveningness' (Archer, et al., 2003; Ebisawa, et al., 2001). Further discussion of the role of '*CLOCK*' genes in relation to sleep quality and diurnal preference is reserved for Chapter 7.

1.6.3 Environmental influences

Whilst evidence clearly implicates genetic factors in sleep and sleep disorders, results from twin studies reveal that genetics alone cannot explain the entire inter-individual variability observed in many phenotypes. In addition to estimating the importance of genetic factors, twin studies estimate the relative importance of shared and non-shared environmental factors on traits or co-occurring traits (see section 1.6.1 for a definition, and Chapter 2 for more information on how these environmental influences are estimated in twin studies). With specific regard to sleep, twin studies in adults have estimated that the remaining source of variance in polysomnographically defined REM sleep and sleep length, and subjective measures of sleep disturbance, insomnia symptoms, daytime sleepiness and sleep quality is due to the non-shared environment with no influence of environmental influences shared between twins (Ambrosius, et al., 2008; Heath, et al., 1990; Hur, et al., 1998; Koskenvuo, et al., 2007; Linkowski, et al., 1991; McCarren, et al., 1994; Vink, et al., 2001; Watson, et al., 2006).

Although typically the twin method does not identify specific environmental influences affecting a phenotype under study (although it is possible to incorporate specific measured environmental effects into more complex models, for example see Gregory, Eley, O'Connor, Rijsdijk, & Plomin, 2005), identification of possible candidate non-shared environmental factors affecting sleep quality can be achieved by looking at research other than quantitative genetics. For example, lifestyle factors such as smoking, drinking alcohol and a lack of exercise have been associated with poor sleep quality and sleep disturbances (Arber, Bote, & Meadows, 2009; Chiu, et al., 1999; Chueh, Yang, Chen, & Chiou, 2009; Hu, Sekine, Gaina, & Kagamimori, 2007; King, Oman, Brassington, Bliwise, & Haskell, 1997; Naylor, et al., 2000; Wetter & Young, 1994). One study found a dose-response relationship between prior smoking and increased risk for developing sleep problems in adolescents (Patten, et al., 2000). In

addition, the experience of stressful life events and socioeconomic status characteristics, including measures such as low household income, low educational attainment, living in rented housing and not being in paid employment, have consistently been associated with sleep disturbances (Arber, et al., 2009; Grandner, et al., 2010; Lavie, 2001; Ohayon, 2002; Sadeh, 1996; Vahtera, et al., 2007). Although these factors may be considered to be 'environmental' in origin, it is possible that they are, in part, influenced by genetic factors – a process termed gene-environment correlation (rGE), which is defined in the following section.

1.6.4 Gene-environment interplay

Although genetic and environmental factors may work independently to some extent, extensive research has investigated the interplay between genetic and environmental influences on a number of emotional and behavioural traits (Rutter & Silberg, 2002). This work has been highly influential with regards to a range of traits such as depression (Caspi, et al., 2003; Eley, Sugden, et al., 2004), and anxiety (Silberg, Rutter, Neale, & Eaves, 2001), yet research assessing the explicit links between genetic and environmental influences focused on sleep is scarce and the research that does exist requires replication before firm conclusions can be drawn. Gene-environment interplay occurs in two forms: gene-environment correlation (rGE); and gene-environment interaction (GxE). These processes are discussed in the following sections and key findings in relation to psychological/behavioural phenotypes in general (given the dearth of research within this area in relation to sleep), from both quantitative and molecular genetic designs, are presented in order to describe these processes within a phenotypic context.

1.6.4.1 Gene-environment correlation

Gene-environment correlation (rGE) is found when genetic effects influence *exposure* to specific environments. As such, it has been suggested that via our genetic propensities we to some extent shape and select our environmental experiences (Plomin, et al., 2008). Gene-environment correlations occur in three forms: passive, active and evocative/reactive. Passive gene-environment correlation refers to the fact that parents provide both genes and environments for their offspring. Thus, the environment provided will be related to the genetic propensities of the parents and consequently, their offspring. Active gene-environment correlation occurs when an individual seeks out environments that correspond to their genotype. For example, an individual who has a genetic predisposition to eveningness (a tendency to get up late in the day and go to bed late at night), may choose a career in which they work in the evening in accordance with their diurnal preference. Evocative/reactive gene-environment correlation occurs when the behaviours performed by an individual based on their genotype elicit certain environmental responses from others (Plomin, DeFries, & Loehlin, 1977). For example, a child who cries at night may evoke a different response from a parent compared to a child who sleeps soundly and causes no disruption, which may consequently affect the family environment.

A robust finding in the literature from twin studies is that the experience of negative life events appears to be in part genetically driven (Bolinskey, Neale, Jacobson, Prescott, & Kendler, 2004; Kendler, Neale, Kessler, Heath, & Eaves, 1993; Plomin, Pedersen, Lichtenstein, McClearn, & Nesselroade, 1990; Thapar & McGuffin, 1996). Furthermore, it has been shown that genetic liability to depression and alcoholism is associated with significantly increased risk of experiencing stressful life events (Kendler & Karkowski-Shuman, 1997). Thus a genetic predisposition to these phenotypes influences exposure to high-risk environments. Further research

demonstrated evidence of genetic correlations between depression and life events – a finding which suggests that the *same* genes were influencing both the phenotype and the environmental risk factor (Lau & Eley, 2008). It is likely that, as in depression, genetically driven exposure to life events is associated with genetic liability to sleep disturbance. Further discussion of this is provided in Chapter 5.

Although explicit research on gene-environment correlation in relation to sleep is currently non-existent, it has been found that there are genetic effects on caffeine consumption (Luciano, Kirk, Heath, & Martin, 2005) - thus there may be evidence here of a gene-environment correlation effect whereby an individual with a genetic predisposition to sleep disturbance may consume more caffeine to counteract the effects of their sleepiness, and so seek out environments where they can drink coffee. In such an instance, the same genes may be influencing the tendency to consume caffeine as those influencing sleep. Indeed, it has been found that there is overlap in the genes influencing coffee-attributed sleep disturbance and sleep disturbance attributed to other factors (Luciano, et al., 2007), suggesting that there may be a correlation between the genetic factors influencing both sleep and the tendency to seek out caffeine.

1.6.4.2 Gene-environment interaction

Whilst gene-environment correlation refers to genetically driven *exposure* to environmental experiences, gene-environment interaction refers to genotype dependent *sensitivity* to high risk environments. What this means is that genetic risk for a particular phenotype is moderated by the presence of an identified environmental stressor. If the genetic propensity to a phenotype is only apparent under certain environmental conditions, ignoring concurrent environmental influences may result in incorrectly concluding that there is little or no genetic influence on that phenotype (Moffitt, Caspi, & Rutter, 2005). Studies estimating GxE thus enable researchers to determine whether

genetic risk is modifiable by exposure to specific measured environmental influences. In the depression literature, several quantitative genetic studies have independently shown that genetic influences on adolescent and adult depression significantly increased in those experiencing negative life events (Kendler, Martin, Heath, & Eaves, 1995; Lau & Eley, 2008; Silberg, et al., 2001). Thus, negative life events pose as a risk factor for the development of depressive symptoms in genetically predisposed individuals. Quantitative genetic studies of GxE in relation to sleep quality, however, are currently non-existent.

In the field of molecular genetics, a finding that has received considerable attention in the depression literature is that the 'short' allele of the *5HTTLPR* gene confers risk for psychopathology in the face of environmental adversity (Caspi, et al., 2003). Although numerous replication attempts of this finding are evident in the literature, there is significant controversy within this field and recent meta-analyses have provided contradictory results. Two meta-analyses claimed that evidence for the interaction between *5HTTLPR* genotype and negative life event exposure in depression was negligible, due in large part to the studies in question being underpowered (Munafo, Durrant, Lewis, & Flint, 2009; Risch, et al., 2009). A more recent meta-analysis, however, found support for this effect, and the authors suggested that the discrepancy between their own and the previous meta-analyses resulted from differences in inclusion criteria (Karg, Burmeister, Shedden, & Sen, 2011). Furthermore, it has been suggested that inconsistencies between replication attempts may be explained by specific sample characteristics and the different methodologies employed to determine environmental adversity (Uher & McGuffin, 2008, 2010).

In terms of molecular genetic research on sleep quality only one study to date has investigated the possibility of GxE. In line with previous work in the field of depression, Brummett and colleagues (2007) demonstrated that individuals carrying one

or two copies of the 'short' allele of *5HTTLPR* had significantly poorer sleep quality assessed by the PSQI than 'long-long' homozygotes – with the effect only significant in individuals experiencing the chronic stress of caregiving for a parent or spouse with dementia. This is evidence of GxE. Although no main effect of genotype on sleep quality was found, this study highlights the need to test for interactions with environmental risk. Failure to do so may result in incorrectly dismissing the role of a particular gene for a given phenotype under investigation if consideration of environmental influences is neglected.

1.7 Associations between Components of Sleep

Although sleep quality may be considered to be a phenotype in its own right research suggests in fact that the construct of sleep quality may encompass several different indices of sleep. As previously mentioned (see section 1.5.2), in the development of the PSQI Buysse and colleagues (1989) identified seven separate 'components' of sleep quality. Some of these components of sleep quality (albeit assessed by a different method) have been shown to co-occur to varying degrees. For example, specifically using the PSQI to assess sleep, Cole and colleagues (2006) found strong correlations between some but not all components, and suggested that certain indices of sleep cluster together more strongly than others. Indeed the authors suggested that, rather than measuring one factor of sleep quality, the PSQI in fact measures 3 separate sleep quality factors. It could be postulated that components that correlate strongly stem from similar aetiological influences. For example, it is possible that genetic factors may influence sleep latency, which may consequently affect sleep duration and quality. Thus, the estimated genetic influence on sleep duration and quality may not be due to independent genetic factors, but rather due to their covariance with

sleep latency – that is, the same genes influence all three phenotypes. Exploration of the overlap in the genetic and environmental influences on the components will determine whether they are similar or distinct, aetiologically. Indeed, de Castro (2002) investigated whether there were independent genetic effects on several indices of sleep-wake behaviour and found that they were largely unique. However, the independence of the environmental influences on the indices of sleep-wake behaviour was not examined. Research is yet to examine the overlap in the aetiological influences on the component clusters encompassed by the PSQI.

Despite 'sleep quality' encompassing different aspects of sleep, sleep quality itself has associations with other indices of normal sleep phenotypes, such as diurnal preference. Specifically, evening-types experience poorer sleep than morning-types (Megdal & Schernhammer, 2007; Ong, Huang, Kuo, & Manber, 2007; Shiihara, et al., 1998; Vardar, Vardar, Molla, Kaynak, & Ersoz, 2008), suffer from greater daytime sleepiness and dysfunction (Vardar, et al., 2008), and display other forms of sleep deficit, such as irregular sleep/wake habits (Taillard, Philip, & Bioulac, 1999). Yet the factors accounting for these associations are yet to be explored. Further discussion of the associations between sleep quality and diurnal preference is reserved for Chapter 6.

1.8 Rationale and Research Questions

The overall aim of this thesis is to gain an understanding of genetic and environmental influences on variation in sleep quality observed in the general population. The research in this thesis uses quantitative and molecular genetic techniques to tease apart the genetic and environmental influences on sleep quality in a sample of young adult twins and siblings from wave 4 of the G1219 study – a large longitudinal population-based twin registry in the UK (for further information, see Chapter 2, section 2.10). There are 5 main aims that this thesis addresses:

1. To investigate the magnitude of the genetic and environmental influences on the components of sleep quality encompassed by the PSQI;
2. To identify specific non-shared environmental influences on sleep quality;
3. To determine the presence of statistical gene-environment correlation and interaction between sleep quality and negative life events;
4. To examine phenotypic and aetiologic associations between sleep quality and diurnal preference;
5. To investigate associations between 3 candidate genetic polymorphisms and sleep quality and diurnal preference, and measured gene-environment interaction with negative life events.

The first set of research questions relate to the first aim of this thesis. Although previous research has highlighted the role of genetic and environmental influences on an overall construct of 'sleep quality' this thesis aims to extend this by breaking this construct down into its constituent components. Furthermore, evidence suggests that the extent to which the components of sleep quality co-occur varies between clusters, however research has failed to address *why* certain components cluster together. Are there specific influences which are shared between components? Knowledge of the overlap in the genetic and environmental influences between phenotypes may be useful for identifying individuals at risk for the development of sleep problems, since identifying an individual with problems in one aspect of sleep may suggest that an individual is genetically sensitive to correlated symptoms. **Chapter 3** discusses these issues and specifically addresses the following 4 questions. To what extent:

(i) do genetic and environmental factors influence global sleep quality measured by the PSQI, as well as the distinct components of sleep quality encompassed by this measure?

(ii) are the components of sleep quality measured by the PSQI correlated?

(iii) is there overlap in the genetic and environmental influences on each of the components of sleep quality assessed separately?

(iv) do genetic and environmental influences account for the associations between components of sleep quality?

The second set of research questions relate to the second aim of this thesis. Previous twin studies have highlighted the importance of the non-shared environment in sleep quality. However, the classical twin method is unable to determine which specific non-shared environmental influences are important for a phenotype. Although previous research from fields other than quantitative genetics have demonstrated associations between sleep and a range of environmental factors, it is possible that these 'environmental' influences are partly influenced by genetic factors – that is, by gene-environment correlation. What this means is that these associations between sleep and seemingly non-shared environmental influences are explained by familial factors, i.e. confounded by the influence of genetics and/or the shared environment. **Chapter 4** aims to identify specific candidate non-shared environmental influences on sleep quality. Using an MZ twin differences design it addresses whether associations with these traditionally viewed 'environmental' factors have a purely non-shared environmental component – that is, whether the associations between sleep and the environment are independent of genetic and shared environmental effects. Specifically this chapter addresses 2 questions:

(i) Are there associations between sleep quality and a range of candidate non-shared environmental factors?

(ii) Is there a purely non-shared environmental component to these associations? i.e. do these associations remain significant even after controlling for genetic and shared environmental factors?

Chapter 5 addresses the third aim of this thesis and investigates the presence of statistical gene-environment interplay between sleep quality and negative life events given the paucity of research investigating this issue to date. Specifically, it determines whether genetic liability to sleep disturbance varies as a function of exposure to negative life events, whilst controlling for the possibility of gene-environment correlation. To this end, this chapter addresses the following 3 research questions:

(i) Are different types of life events associated with sleep quality differentially?

(ii) To what extent do genetic and environmental factors influence negative life events?

(iii) Does the presence of negative life events moderate genetic liability to sleep disturbance, controlling for gene-environment correlation?

Chapter 6 investigates the association between sleep quality and diurnal preference outlined in the fourth aim of this thesis. While there appears to be an association between diurnal preference and sleep quality as indicated by previous research, what is currently unclear is what accounts for this association. It is of interest to determine whether there is similarity in the genetic influences between sleep quality

and diurnal preference as such information has the potential to inform molecular genetic research in the search for genes influencing different aspects of sleep. If genetic overlap between phenotypes is found, genes already known to influence one phenotype may be worthy candidates for exploration with regards to other phenotypes with which it is associated. If, however, genetic overlap is small, this would imply that unique genes should be sought with regards to the phenotypes under study. As such, this chapter addresses the following 4 questions:

(i) To what extent do genetic and environmental factors influence diurnal preference?

(ii) How strong is the association between sleep quality and diurnal preference in the G1219 sample, and is this in line with previous reports?

(iii) Is there overlap in the genetic and environmental factors influencing sleep quality and diurnal preference?

(iv) To what extent do genetic and environmental influences account for the association between sleep quality and diurnal preference?

The fifth aim of this thesis is addressed in **Chapter 7**. Given the dearth of research investigating specific genetic variations in aspects of subjective sleep quality, this section of the thesis takes a candidate gene approach to examine 3 genetic polymorphisms (*5HTTLPR*, *PER3* and *CLOCK* 3111) in relation to sleep quality and diurnal preference. Evidence of the overlap in the genetic influences between these phenotypes from Chapter 6 directed the search for specific candidate genes that may be influential to both of these phenotypes. For example, genes associated with sleep quality, such as serotonin, may be worthy candidates for investigation with regard to diurnal preference, given the genetic overlap between these phenotypes. As such, these

3 genetic polymorphisms were selected because research has previously implicated them in either sleep quality or diurnal preference. Furthermore, previous research has highlighted the importance of investigating the possibility of gene-environment interactions in molecular genetic research (Moffitt, et al., 2005). Accordingly, investigation of GxE with negative life events is incorporated into the analyses, given the evidence for GxE with this environmental risk factor in other psychiatric disorders, and the paucity of research assessing GxE in sleep phenotypes. Although GxE is also investigated statistically in Chapter 5, incorporating measured GxE in molecular studies is considered to have greater power. In addition, it is useful to investigate the presence of gene-gene interactions (epistasis). Previous studies have suggested that the interaction of genes may contribute to complex human psychiatric disorders (Murphy, et al., 2003), and that investigating combinations of genes may be more informative and accurate than investigating single genes in isolation (Pedrazzoli, et al., 2010). However, little is known about the possibility of epistatic mechanisms in sleep and the polymorphisms under study here. Specifically, **Chapter 7** addresses the following 4 research questions:

(i) Is there evidence of a main effect of *5HTTLPR*, *PER3* and/or *CLOCK* 3111 on sleep quality?

(ii) Is there evidence of a main effect of *5HTTLPR*, *PER3* and/or *CLOCK* 3111 on diurnal preference?

(iii) Are there significant interactions between these genetic polymorphisms and environmental risk factors for both sleep quality and diurnal preference (GxE)?

(iv) Are there significant gene-gene interactions (GxG) on sleep quality and diurnal preference?

Additionally, throughout this thesis sex differences are examined. Specifically, all analyses consider whether there are significant differences between males and females in absolute sleep scores, as well as whether the sexes differ in terms of the aetiological influences on the phenotypes under study. Although it would also be beneficial to examine age differences with regards to both phenotypic and aetiologic results, the age spread of participants included in the study was homogeneous, and so developmental questions are not addressed in this thesis. As such, all results describe the sleep of young adults (aged between 18 and 27 years; 90% of participants aged 18-22 years). As can been seen from the above aims, the research encompassed in this thesis fully utilises the twin design to answer a range of questions regarding the aetiology of sleep quality using a variety of quantitative and molecular genetic techniques.

Chapter 2: Methodology

2.1 Overview

This chapter begins by outlining the basics of quantitative genetic analyses using MZ and DZ twins and siblings in order to investigate genetic and environmental influences on traits. Following this is a description of the assumptions of the twin method, and detailed model-fitting procedures for univariate, multivariate and sex-limitation models using structural equation modelling. This will be relevant to Chapters 3, 5 and 6, where basic univariate and multivariate models (in addition to more complex models) will be used. This chapter will describe only models that will be relevant throughout the thesis. Where more complex models are used detailed information on these models is reserved for the appropriate chapter. Accordingly, Chapter 3 will describe in detail threshold liability models, which are a reformulation of the univariate analyses described in the current chapter for use with categorical data. Chapter 4 will describe MZ twin differences analysis, which is a method used to identify purely non-shared environmental components to associations between the environment and sleep. Chapter 5 will describe models of statistical gene-environment interaction in the presence of gene-environment correlation. After describing the twin method, the current chapter will outline the basics of molecular genetic analysis and genotyping techniques which will be relevant to Chapter 7, followed by a description of the sample used throughout this thesis.

2.2 Introduction

As mentioned previously (see section 1.6.1), classical twin studies rely on the differences in genetic relatedness between monozygotic twins, who share 100% of their genetic make-up, to dizygotic twins who share on average only 50% of their segregating

genes to make inferences about the aetiology of phenotypes. Because the genetic relatedness of DZ twins is the same as that of full siblings, quantitative genetic studies may also incorporate data from full siblings in order to estimate the relative proportions of genetic and environmental influences on phenotypes. Quantitative genetic studies use these differences in genetic relatedness to estimate 2 forms of genetic influence: additive and non-additive. As described in Chapter 1 (section 1.6.1) additive genetic influence can be described as the cumulative effect, or the 'adding up', of genes to influence behaviour. In pairs of MZ twins, the additive genetic correlation between each twin for any trait is assumed to be 1, since they inherit exactly the same genetic information from their parents, and so share 100% of their genes. In DZ twins and siblings, this is estimated to be 0.5, since they are assumed to share 50% of their segregating genes. Non-additive genetic influence, otherwise referred to as 'dominance', describes not the 'adding up' of genes, but rather the combination or *interaction* of alleles at a given locus which influence behaviour (this is in contrast to epistasis which is the interaction of alleles at different loci). In MZ twins, the correlation between twins due to dominance is expected to be 1, since their genetic make-up is identical, whereas for DZ twins, this is estimated to be 0.25. This is because individuals receive only one of each of a pair of alleles from their parents rather than a combination, and so the combination of alleles between parents and offspring are entirely different. However, siblings are expected to receive the same combination of alleles as each other from their parents one-fourth of the time (Vink, et al., 2001). A common way of describing these types of genetic effects is *narrow-sense* and *broad-sense* heritability. *Narrow-sense heritability* refers to only the proportion of the phenotypic variance accounted for by additive genetic factors. *Broad-sense heritability*, however, refers to all of the genetic effects, additive and non-additive, that account for the phenotypic variance.

In addition to calculating genetic influences, quantitative genetic studies estimate 2 forms of environmental influence: shared and non-shared environmental influences. Shared environmental influences are those that act to make family members similar. Because environmental influences are unrelated to genetic relatedness, correlations between MZ and DZ twins due to the shared environment are the same, and are assumed to be 1. This is because the definition of the shared environment is that it is entirely shared between the twins. In children an example of the shared environment may be diet or attending the same schools, the influences of which are commonly shared between siblings. However, in adult twins or siblings who no longer live together, shared environmental influences are often negligible for most behavioural phenotypes. It is a common theme in behavioural genetic research that the shared environment becomes increasingly less important with increasing age, with the consequence that genetic influences become more important (McGue, 2008). It is proposed that this observation is explained by the fact that, with increasing age, individuals have greater control over their environmental experiences – and that the experiences they choose are correlated with their genetic propensities (Scarr & McCartney, 1983). This effect has been shown to be strongest in the transition from adolescence to young adulthood (Bergen, Gardner, & Kendler, 2007). Furthermore, this observation may be largely due to the increasing importance of environmental experiences that occur outside of the family environment as one gets older (Plomin, et al., 2008). Such influences are called the non-shared environment which can be described as environmental influences unique to each twin/sibling which account for their dissimilarity. Correlations within pairs of MZ and DZ twins for this source of variance are expected to be zero. An example of which could be one twin being in a motor vehicle accident.

2.3 Assumptions and Considerations of Twin Studies

Although twin studies reliably provide useful information about the aetiology of a trait (or traits) of interest, twin studies rest on several assumptions that, if violated, may lead to incorrect estimates of the relative contribution of genetic and environmental influences. These assumptions and considerations include assortative mating, zygosity determination, equal environments and generalisability.

2.3.1 Assortative mating

A fundamental assumption of the twin method is that parental mating in the population is random. If this is true, DZ twins will share on average 50% of their segregating genes – a central tenet of the twin method. Assortative mating refers to the process of non-random mating whereby individuals choose partners similar to themselves on particular traits, i.e. for like to marry like (positive assortative mating), or for individuals to choose partners dissimilar from themselves, where 'opposites attract' (negative assortative mating). In such cases, DZ twins would be more (positive assortative mating) or less (negative assortative mating) similar than would be expected if mating were random. Positive assortative mating has the effect that genotypic variance for a particular trait in the population is increased over generations. This means that for the particular trait in question, offspring differ from the population mean to a greater extent than would occur if mating were random. In family studies, positive assortative mating increases correlations between first-degree relatives (parent-offspring) with the consequence that heritability estimates would be inflated. However, in twin studies heritability estimates would be underestimated in the presence of assortative mating. This is because correlations between DZ twins would be inflated since they have the same genetic relatedness as first-degree relatives, whereas MZ twin

correlations would not be affected since they are 100% genetically identical. Thus, the differences in correlations between MZ and DZ twins would be reduced, with the consequence that heritability estimates would be lowered and estimates of the shared environment overestimated (Plomin, et al., 2008).

Assortative mating has been found to be present for a number of psychiatric difficulties (Maes, et al., 1998). One method to assess the presence of assortative mating is to inspect data from parents on the trait of interest and to control for this in genetic modelling (Neale & Cardon, 1992). However, its effects in models of heritability and gene-environment interaction have been found to be negligible (Loehlin, Harden, & Turkheimer, 2009; Maes, et al., 1998) which suggests that although consideration of assortative mating is worthy, violations of this assumption are not problematic in twin studies in most cases.

2.3.2 Zygosity determination

Because the twin method hinges on the differences between MZ and DZ twins, correct identification of twin zygosity is essential. One method to determine zygosity is to examine DNA markers. If there are any DNA marker differences between twins within a pair, they must be DZ twins. If no differences are found, they must be MZ twins. Although this is an accurate method of determining zygosity, and has indeed been shown to yield around 99% accuracy when assessing just 4 loci (Chen, et al., 2010), it may be costly and time consuming to collect and analyse DNA for the purposes of zygosity assessment in twin studies (which typically analyse phenotypic differences and infer from twin correlations information regarding genetics). An alternative method to assess zygosity is to ask parents questions about the physical similarity of their twins (for example eye or hair colour). The questionnaire method is

standard in twin studies and has been shown to have ~95% accuracy in correctly determining zygosity when validated against DNA markers (Price, et al., 2000).

2.3.3 Equal environments assumption

The equal environments assumption is an important consideration of the twin method. It assumes that the shared environments experienced by MZ and DZ twins are shared to roughly the same extent between the two different types of twins. Such an assumption implies that the only reason why MZ twins are more similar to one another than DZ twins is because of their differences in genetic relatedness and not due to them experiencing more similar environments. Violations of this assumption would mean that estimates of genetic influence on a trait would be artificially inflated (Plomin, et al., 2008). In children, it has been shown that MZ twins often dress alike, share the same friends and (of particular relevance to this thesis) share bedrooms more often than DZ twins (Loehlin & Nichols, 1976). However, tests of the equal environments assumption for a variety of traits by examining environmental similarity, for example treatment similarity, physical similarity, and frequency of contact in adulthood, have concluded that the assumption is not violated in such cases (Derks, Dolan, & Boomsma, 2006). Another method of testing the equal environments assumption is to examine twins mislabelled as MZ or DZ by themselves and others. If indeed MZ twins are treated more similarly than DZ twins, DZ twins incorrectly labelled as MZ twins should be more similar than expected if the equal environments assumption is violated. Likewise, MZ twins incorrectly labelled as DZ twins should be less similar in violations of the assumption. Specific tests of this in mislabelled twins, has however provided no evidence that perceived zygosity has an influence on similarity in a number of psychiatric disorders (Kendler & Gardner, 1998).

A common objection to the equal environments assumption is that it is incorrectly believed that MZ twins have more similar prenatal environments than DZ twins because they often share the same chorion (sac in the amnion). However, MZ twins may in fact experience greater *differences* prenatally than DZ twins, since each twin is in competition with their co-twin for placental nutrients. Evidence for this claim can be found from studies demonstrating that MZ twins who share a chorion have lower birth weight, higher incidence of morbidity, and are more likely to suffer birth defects than dichorionic twins (Adegbite, Castille, Ward, & Bajoria, 2004; Gonzalez, et al., 2010). If MZ twins do experience less similar environments prenatally, this would have the effect that heritability estimates would be underestimated in such instances.

2.3.4 Generalisability

A common criticism of twin studies centres on the fact that twins may not be representative of the general population. If this is true, then making assumptions about members of the general population based on the findings from twin studies may be misguided. There are several differences between twins and singletons. Twins are often born prematurely, have a much lower birth weight, and are more likely to suffer from congenital malformations, obstetric complications and perinatal mortality, than singletons (Rutter & Redshaw, 1991). It has also been suggested that the rearing environments of twins may differ somewhat from singletons, and that these differences may lead to developmental complications, such as delayed language development (Rutter & Redshaw, 1991). However, a comparison of twins and non-twins in terms of risk for psychiatric symptoms, including insomnia, demonstrated that there were no consistent twin-singleton differences in symptomatology (Kendler, et al., 1995), which suggests that for such factors twins may well be representative of the general population.

2.4 Univariate Genetic Analysis

At the most fundamental level quantitative genetic analysis uses the intraclass correlation coefficient (*r*) to assess the degree of relationship between two quantitative variables when these measurements are organised into groups, i.e. when measured in pairs of MZ/DZ twins. The intraclass correlation coefficient differs from the standard Pearson correlation coefficient as it is calculated by centring the means and standard deviations of scores within each pair of observations pooled by group, rather than centring the data for each pair of observations individually (as in the standard Pearson correlation). Accordingly, the intraclass correlation coefficient is defined as:

$$r = \frac{MSB - MSW}{MSB + MSW} \quad (2.1)$$

Where MSB is the mean-squares between pairs, and MSW is the mean squares within pairs (McGue & Bouchard, 1984). Throughout this thesis, intraclass correlations are reported unless otherwise stated. Of note, the magnitude of all correlations reported in this thesis are considered according to the criteria set out by Cohen (1988), where a correlation of 0.1 is considered small, 0.3 = medium and 0.5 = large.

Using the intraclass correlation between pairs of MZ and DZ twins and siblings on a quantitative trait of interest, univariate genetic models are used to estimate the contribution of additive genetic (A), non-additive genetic (D) *or* shared environmental (C), and non-shared environmental (E) (which includes measurement error) components of variance on a single (univariate) trait. It is not possible to model both non-additive genetic effects and shared environmental effects simultaneously because C and D predict different MZ and DZ twin correlation ratios, and the effect of both is confounded if examined together (Neale & Cardon, 1992). Thus, these effects are

examined in separate models (i.e., either by an ACE or ADE model) as appropriate. The sum of these sources of variance equals the total phenotypic variation in the trait (V_P):

$$V_P = A + C + E \quad (2.2)$$

or

$$V_P = A + D + E \quad (2.3)$$

The relative proportions of A, D *or* C and E are calculated using Falconer's formula (Falconer & MacKay, 1996) which uses the differences in the intraclass correlation coefficients (*r*) between MZ and DZ twins and siblings. For an ACE model, similarity between MZ twins (*r*MZ) for a particular trait is accounted for by genetics and shared environment, so that:

$$r\text{MZ} = A + C \quad (2.4)$$

Since MZ twins share 100% of their genetic make-up and their shared environment, both A and C equal 1. For the similarity in DZ twins (*r*DZ), genetics and shared environment are important, but as DZ twins share only 50% of their segregating genes A is estimated to be half, so that:

$$r\text{DZ} = ½\ A + C \quad (2.5)$$

In ADE models, *r*MZ remains the same since A and D are both expected to be 1, however, the calculation for *r*DZ is amended to reflect the fact that the proportion of the same alleles received from parents due to non-additive effects occurs only one-fourth of the time between siblings:

$$r\text{DZ} = ½\ A + ¼\ D \quad (2.6)$$

Using these twin correlations it is possible to calculate the relative proportions of genetic and environmental influences on a trait. For the ACE model, additive genetic influence is calculated as twice the difference in the MZ and DZ twin correlations:

$$A = 2(rMZ - rDZ) \quad (2.7)$$

Shared environment is calculated as the difference between the MZ correlation and additive genetics:

$$C = rMZ - A \quad (2.8)$$

As the only reason MZ twins differ is due to non-shared environmental effects, the influence of the non-shared environment is easily calculated as the total phenotypic variance minus the MZ correlation:

$$E = 1 - rMZ \quad (2.9)$$

Because these equations use correlations between different types of twins (which are standardized covariances), the estimates are standardized so that the total phenotypic variance always equals 1. Thus, A, C and E represent relative proportions of variance.

It is not possible, however, to calculate the effects of non-additive genetic effects in this way. In such instances, more complicated calculations are performed in structural equation modelling programmes, described below. The decision as to whether to examine an ACE or an ADE model rests on the observed MZ/DZ correlation ratios. If the MZ twin correlation is more than double that of the DZ twins, non-additive genetic influences may be important for the trait under study.

It is also important to note that it is likely that the parameter estimates derived from formal model fitting procedures (as described below) may not provide exact estimates as those calculated by hand using Falconer's formulae. This is because

structural equation modelling uses more information than just the simple correlations, and because it requires additional assumptions based on the variances of the raw data.

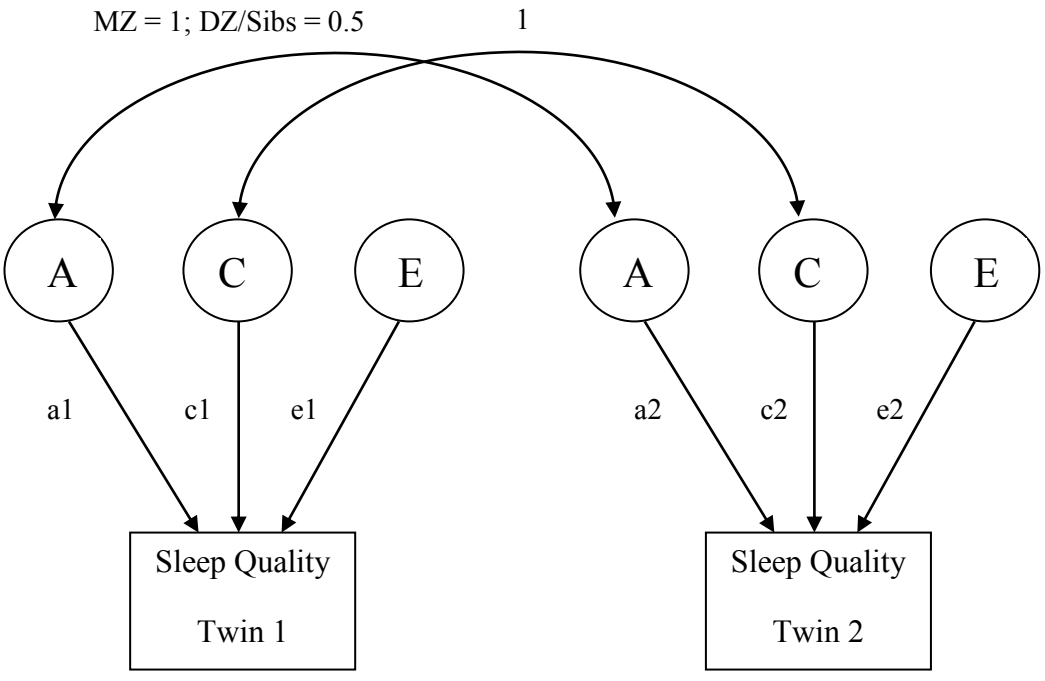
2.5 Path Diagrams

Path diagrams are a method used in twin research to describe genetic analyses pictorially. The purpose of path diagrams is to illustrate the resemblance between twins and the relationships between different factors in structural equation models, which is used to help workout the algebraic formulae. Shown in **Figure 2.1** is a standard path diagram for a univariately assessed trait (sleep quality) for one twin pair. In path analysis, there are several conventions and rules that facilitate interpretation of the diagram. Rectangles represent measured variables, circles indicate latent (estimated) parameters, single headed arrows indicate causal paths, and double-headed arrows represent correlations between variables. With regard to **Figure 2.1** 'sleep quality' is a measured trait and so is depicted by a rectangle. The genetic, shared and non-shared environmental variance components, A, C and E, are latent variables as they are estimated by the model and so are represented by circles. The downward arrows resemble the partial regression coefficients for A, C and E and represent their causal relationship with sleep quality. The double-headed arrows represent the standardised covariance (correlation) between the latent variables between the two twins. For MZ twins, the standardised covariance for additive genetics, A, is 1 as they are genetically identical, whereas for DZ twins this is 0.5. The shared environmental standardised covariance, C, is 1 for both types of twins. There is no double-headed arrow for the non-shared environment, E, as this source of variance is not shared between family members.

In addition to these conventions there are path tracing rules which make it possible to calculate the variances for individual variables and the covariances between

multiple variables. To calculate the covariance between twin 1's sleep quality and twin 2's sleep quality, a path must be traced from the first variable to the second variable, using the following three rules: First, it is possible to move forward along a path, but not forward and then backward. Second, each variable may only be passed through once, and third only one double-headed arrow may be traced in one path. The product of the path tracing is then summed. Thus, the standardised covariance in sleep quality between MZ twins can be calculated by summing the following paths: (a1 x 1 x a2) + (c1 x 1 x c2), which can also be described as $a^2 + c^2$, or A + C. For DZ twins, the standardised covariance in sleep quality is calculated as: (a1 x 0.5 x a2) + (c1 x 1 x c2), which can also be described as $0.5a^2 + c^2$, or 0.5A + C.

Figure 2.1. Standard path diagram for a univariate ACE model for one twin pair.



Note. MZ = Monozygotic; DZ = Dizygotic; Sibs = siblings; A = Additive genetic influence; C = Shared environmental influence; E = Non-shared environmental influence; a1, c1, e1 = aetiological influences on twin one; a2, c2, e2 = aetiological influences on twin 2.

2.6 Structural Equation Modelling

Although it is possible to calculate the relative proportions of genetic and environmental influences on traits using Falconer's equations, it is advantageous to perform more formal model fitting techniques. One advantage of such model fitting is that it is possible to determine whether the ACE model is in fact a true representation of the underpinnings of a particular trait, or whether an alternative model, i.e. an ADE or AE model, is more appropriate. A second advantage is that calculation of confidence intervals around the parameter estimates is possible, which gives a direct indication of the significance of the variance components. Additionally, it is possible to investigate co-occurring traits, sex differences, and models of gene-environment correlation and interaction - analyses which will be discussed in sections 2.7, 2.8 and Chapter 5, respectively.

2.6.1 Model-fitting information

In model-fitting analyses, structural equation models are constructed by incorporating certain parameters, for example the variance components, into a model which are used to describe the observed twin/sibling data. The observed data takes the form of variance-covariance matrices for the different twin/sibling pairs for a particular trait. Model-fitting involves estimating different values for the model parameters, which will consequently produce different variance-covariance matrices, and aims to determine the most parsimonious model, i.e. one that incorporates the fewest parameters, that most accurately generates expectations in line with those in the observed data (Plomin, et al., 2008). Because of the iterative and computationally intensive nature of model fitting, computer software has been designed to compute these calculations.

The most widely used statistical package to analyse genetically sensitive data is Mx (Neale, 1997), which performs structural equation modelling using maximum-likelihood estimation, and accounts for the non-independence of twin data. The fit statistic provided by Mx for raw data modelling is -2LL (minus twice the log likelihood of the observations). The -2LL value, in itself, provides no information on fit, however, there are two main indices of fit that are produced by Mx that can be used to determine the best-fitting model. The first is the chi-square (χ^2) goodness-of-fit statistic. This can be used to directly test the significance of the differences between two models to determine which provides the best approximation of the observed data. The second is the Akaike Information Criterion (AIC) which accounts for the number of parameters being estimated and goodness-of-fit - thus more parsimonious models are favoured by the AIC. AIC is calculated as the change in χ^2 between two models minus twice the change in degrees of freedom:

$$(\Delta\chi^2 - 2 \times \Delta df) \tag{2.10}$$

Good fit is indicated by low χ^2 statistics and low, negative AIC values.

2.6.2 Model-fitting procedures

Model-fitting begins by fitting a saturated model to the data which estimates the maximum number of parameters required to describe the variance-covariance matrix and means of observed variables, and thus provides a perfect fit to the data. Genetic models, which typically include the model parameters A, C and E, are then approximated to the data. The -2LL of the saturated model is then subtracted from the -2LL of the genetic model. As mentioned previously, the -2LL value, in itself, provides no information on fit, however the difference between -2LL for the saturated and genetic models is χ^2 distributed, and so provides a relative fit of the data. A non-

significant difference in fit between the genetic and saturated models indicates that the genetic model does not fit the data less well than a saturated model and therefore provides a good description of the data. Fit of the model is also tested by observing the AIC statistic as outlined above. Alternative models (for example, an ADE model) are then approximated to the data, the fit of which is compared again to the saturated model. Additionally, it is possible to fit *nested* models within the genetic models which constrain at least one of the estimated parameters (for example, C) to zero. In so doing, the parameter in question is dropped from the model in order to determine whether its exclusion reduces the fit of the model. Parameters which can be dropped without a significant reduction in fit of the model compared to the fuller model are excluded from the final model in order to adhere to parsimony. Likelihood-based 95% confidence intervals (CIs) on the parameter estimates are then obtained in order to determine their precision.

2.7 Multivariate Genetic Analysis

In order to answer questions regarding multiple phenotypes simultaneously it is necessary to expand the standard univariate genetic model to the multivariate case. In univariate analyses we estimate the cross-twin/sibling same-trait correlations in pairs of MZ twins, DZ twins and non-twin siblings, separately (e.g. sleep $\text{quality}_{\text{twin1}}$ and sleep $\text{quality}_{\text{twin2}}$). The difference in similarity between these groups is used to estimate genetic and environmental influences upon traits as modelled in the univariate genetic analyses. In addition to this however, multivariate analyses also use the within-twin cross-trait (e.g. sleep $\text{quality}_{\text{twin1}}$ and sleep $\text{duration}_{\text{twin1}}$), and the cross-twin cross-trait (e.g. sleep $\text{quality}_{\text{twin1}}$ and sleep $\text{duration}_{\text{twin2}}$) covariances, measured between pairs of MZ and DZ twins and non-twin siblings, separately, to assess the aetiological factors influencing the relationships between phenotypes. While significant within-twin/sibling

cross-trait correlations imply common aetiological influences, the power to distinguish between different sources of variance causing the correlation is derived from the cross-twin/sibling cross-trait correlations. Significant cross-twin/sibling cross-trait correlations imply that these common aetiological influences are familial. Whether these familial influences are genetic or environmental in origin, is indicated by the MZ:DZ/sibling ratio of these correlations. If the association between traits in MZ pairs is greater than that of DZ/sibling pairs, additive genetic influences (A) are implied. If, however, the MZ pair association is similar to that of the DZ/sibling pairs, shared environmental influences (C) are implied. Non-significant cross-twin/sibling cross-trait correlations imply that the common aetiological influences on the phenotypes are due to the non-shared environment (E), not familial effects.

There are several multivariate models that can also be used to formally test the aetiological associations between multiple phenotypes, for example, common and independent pathways models (Rijsdijk, 2005), cholesky decomposition and correlated factors models (Neale & Cardon, 1992). The correlated factors model is mathematically equivalent to the Cholesky model, however, it makes no assumptions as to the direction of the effects and so is appropriate in studies where there are no *a priori* hypotheses regarding causality (Loehlin, 1996). The decision as to which model to use is dependent on the research questions and hypotheses that one wishes to test (or of course all can be run to see which fits the data best). The *bivariate correlated factors model* (which can be extended to include several phenotypes if required), not only allows us to answer questions about the extent of genetic and environmental influences on each of the phenotypes included in the model, but it also allows the influences of one phenotype to correlate with those of the other phenotype to inform us about the relationships between them. Specifically, it allows us to determine: (i) how well two phenotypes are correlated; (ii) the extent to which the genetic and environmental influences on the

phenotypes overlap, that is, how similar the variance components are between phenotypes; and (iii) to what extent genetic and environmental influences explain the phenotypic correlation. Thus, this model provides information as to whether there are shared aetiological factors between phenotypes. **Figure 2.2** illustrates a bivariate correlated factors model for two measured phenotypes: sleep quality and sleep duration. This diagram is shown for one twin only. There are three new parameters in this diagram, *rA*, *rC* and *rE*. These represent the additive genetic, shared environmental and non-shared environmental correlations between the phenotypes. These give an indication as to the overlap in these influences, i.e. the extent to which they are the same between the phenotypes. For example, an additive genetic correlation of 1 would indicate that exactly the same genes are responsible for sleep quality and sleep duration. Such knowledge would be useful for identifying specific genes associated with these phenotypes, since if a gene is known to be associated with, for example, sleep latency, knowledge of the genetic overlap between sleep latency and sleep duration implies that the same gene should be sought with regards to sleep duration. An additive genetic correlation of zero would indicate that entirely different genes influence the phenotypes. The same reasoning can be applied to the shared and non-shared environmental components. For example, a non-shared environmental correlation of zero would indicate that the environmental influences that make twins within a pair differ on sleep latency are entirely independent of those on sleep duration. Using this information (and using sleep latency and sleep duration as examples), it is possible to calculate the proportion of the phenotypic association accounted for by additive genetic influences, as:

$$\sqrt{A}_{\text{sleep latency}} \text{ X } rA \text{ X } \sqrt{A}_{\text{sleep duration}} \text{ / phenotypic correlation} \qquad (2.11)$$

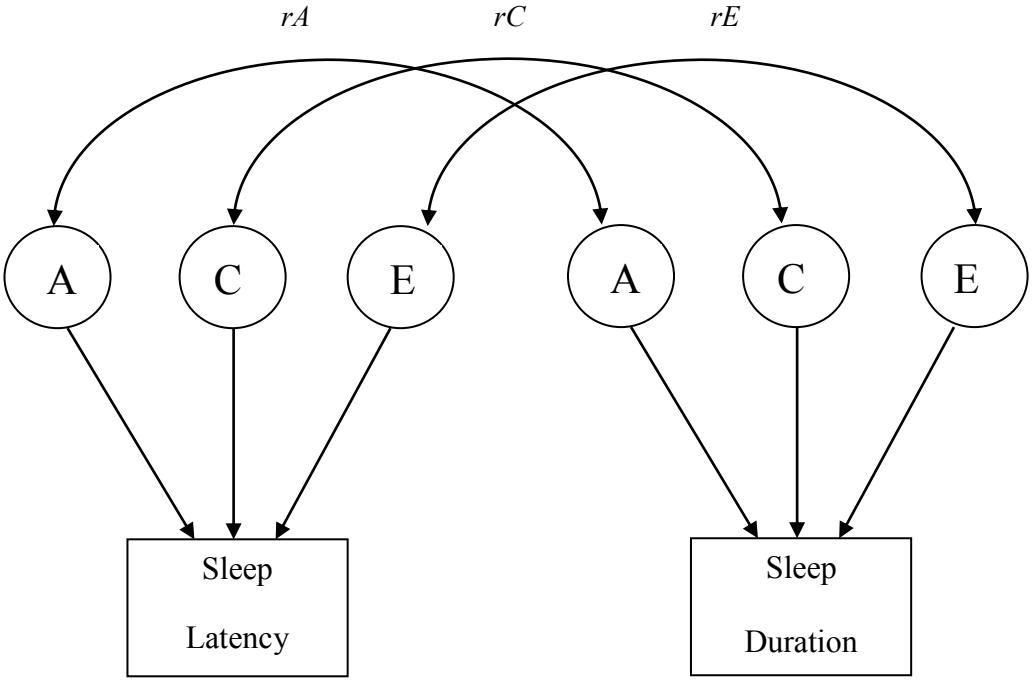
Note that the square root of the univariate *A* path (for both phenotypes) is used as, in the bivariate case, we only trace up this path once (whereas in the univariate case we would trace up and then back down the path – squaring the estimate), thus deriving the unsquared estimate. Likewise the proportion of the phenotypic association accounted for by shared environmental influences can be calculated as:

$$\sqrt{C}_{\text{sleep latency}} \text{ X } rC \text{ X } \sqrt{C}_{\text{sleep duration}} \text{ / phenotypic correlation} \quad (2.12)$$

And finally the proportion of the phenotypic association accounted for by the non-shared environment can be calculated as:

$$\sqrt{E}_{\text{sleep latency}} \text{ X } rE \text{ X } \sqrt{E}_{\text{sleep duration}} \text{ / phenotypic correlation} \quad (2.13)$$

Figure 2.2. Path diagram for a bivariate correlated factors model shown for one twin only.



Note. A = Additive genetic influence; C = Shared environmental influence; E = Non-shared environmental influence; *rA* = Additive genetic correlation; *rC* = Shared environmental correlation; *rE* = Non-shared environmental correlation.

2.8 Sex-Limitation Models

In addition to assessing the aetiological contributions on phenotypes or the co-occurrence between phenotypes, twin analyses using both male and female twin pairs are also able to determine whether there are various types of sex differences. There are three different types of sex differences that can be investigated in model-fitting analyses: *quantitative*, *qualitative* and *scalar*. *Quantitative sex differences* refer to the extent to which the magnitude of genetic and environmental influences differs between males and females. This can be tested by estimating the proportions of genetic and environmental influences for males and females separately, and then equating the estimates and observing the consequent change in fit of the model. If constraining the estimates to be equal between males and females results in a significantly worse fit compared to a model in which the estimates are free to vary between the sexes, there is evidence of a statistically significant quantitative sex difference. *Qualitative sex differences* refer to aetiological influences on a phenotype differing for males and females. It is possible to test, for example, whether the additive genetic influences are the same between the sexes, by constraining the additive genetic correlation between males and females to be 1 (indicating that exactly the same genes are important for the phenotype in males and females alike) and comparing the resulting fit to a model in which the correlation is free to vary. If the constrained model results in a significantly worse fit, this suggests evidence of qualitative sex differences (that is, that different sets of genes are important for males and females), the magnitude of which can be assessed by observing the additive genetic correlation between the sexes. *Scalar sex differences* refer to the differences in variance for a phenotype between males and females. This can be tested by constraining the parameter estimates to be equal between males and females, but allowing a phenotypic variance difference by incorporating a multiplicative

scalar to account for proportional differences between the sexes. The fit of this model is then compared to simpler, non-scalar models.

2.9 Molecular Genetic Genotyping Techniques

Whilst twin studies provide useful information regarding the extent to which genes and environments may contribute to a trait or co-occurring traits, and determine the degree of overlap in the aetiological factors between phenotypes, they tell us nothing about the specific genes that may be important for such phenotypes. In order to answer questions related to the specific influence of certain genes it is necessary to perform tests of association by analysing segments of DNA and relating differences in specific genes to phenotypic differences between individuals. This section begins by describing the basics of molecular genetics followed by details of genotyping techniques using polymerase chain reaction, and methods of visualisation.

2.9.1 Basics of molecular genetics

DNA is a molecule which contains the genetic information required to synthesize proteins. DNA molecules are made up of 4 nucleotide bases: adenine (A), guanine (G), cytosine (C) and thymine (T). The nucleotides form pairs, the A base with T, and the C base with G, so that the double-helix structure of DNA contains two strands of nucleotide sequences which are entirely complimentary to one another. Long segments or sequences of these nucleotide bases are organised into genes – the basic units of heredity. Genes often come in two or more forms, called alleles, which occur at a particular locus. We receive one allele of a particular gene from each of our parents, and within an individual these alleles may be identical, in which case the gene is said to be homozygous, or non-identical and called heterozygous. Small variations (known as

polymorphisms) in the structure of the segments of genes made up of the nucleotides are often associated with phenotypic variations observed in the population. This is because it is likely that different polymorphisms lead to the production of different amino acids when the gene is translated into a protein. However, it should be noted that it is also likely that phenotypic effects may be associated with non-functional (i.e. non-protein coding) polymorphic regions of DNA. It is these variations (in both functional and non-functional coding regions) that are the focus of molecular genetic association studies.

There are several different types of polymorphisms, some of which involve just one nucleotide and are hence called 'single nucleotide polymorphisms' (SNPs). SNPs come in various forms: *substitution*, *deletion*, and *insertion. Substitution* refers to the change in a sequence of the nucleotides often of a single base (for example an A base substituted for a G), whereas *deletion* refers to the loss of a particular base, and *insertion* to the gain of an additional base, within a sequence. Although sometimes such variations have no (or no known as yet) function, some polymorphisms have been found to have a functional role. For example, a single base substitution in the *CLOCK* gene of mice reduces transcriptional activity of its protein products resulting in the deletion of 51 amino acids. This has the consequent effect of lengthening the locomotor activity rhythm period in mice homozygous for the mutant *CLOCK* allele by around 3-4 hours (King, Zhao, et al., 1997). Other types of polymorphisms involve structural rearrangements such as *duplication, length variations,* or *variable number tandem repeats* (VNTR) of microsatellites (short tandem repetitive sequences that contain between 2-5 nucleotides, and vary in length between individuals (Weir, Anderson, & Hepler, 2006)). *Duplication* refers to the duplication of a specific segment of DNA. *Length variations,* however, occur when particular segments are repeated a variable number of times (as such length variations could be considered to be

insertions/deletions, where a particular sequence is either missing or added). An example of this is the length variation consisting of a 'short' or 'long' allele in the transporter region of the serotonin gene (*5HTTLPR*) associated with primary insomnia (Deuschle, et al., 2010). A *variable number tandem repeat* however, is when a small segment, usually a combination of 2 or more nucleotides, is repeated a variable number of times. For example, a VNTR in *PER3* contains a 54-nucleotide coding region sequence which is repeated either 4 or 5 times, and has been found to be associated with diurnal preference (Archer, et al., 2003).

2.9.2. Polymerase chain reaction

Identification of genetic variants is usually carried out using *polymerase chain reaction* (PCR) based techniques. PCR is a process whereby small segments of DNA (usually around 200-1000 base pairs) are replicated exponentially so that the segments are amplified for observation in the laboratory (O'Donovan & Owen, 2002). PCR requires several ingredients or *reagents*. The segment of DNA of interest (the template) is first identified, and a pair of primers (synthetic single stranded DNA molecules) are chemically synthesized. One primer is identical to the 5' end of the genomic DNA sequence to be replicated, the other to the complimentary strand of DNA. The primers act as starting points for DNA replication by isolating the region of interest. In addition to the DNA template and primers, PCR requires a mixture of deoxynucleoside triphosphates (dNTPs) – the individual 4 nucleotides that make up DNA, and *Taq polymerase*, an enzyme which synthesizes new strands of DNA.

PCR involves heating this mixture in a thermocycler at varying temperatures. First, during the *denaturation* stage, the DNA is heated to around 95°C for roughly 30 seconds so that the double stranded DNA is split into two single strands. Second, in a process called *hybridization* the mixture is cooled to around 50°C for roughly 30

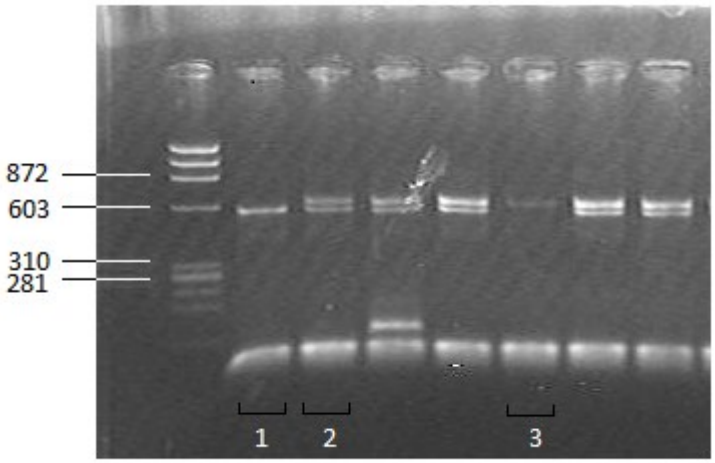
seconds so that the primers *anneal* to each of the separate strands of DNA by base pairing. Third, the taq polymerase attaches to the primer segments ready for DNA synthesis. Fourth, in the *extension step* the mixture is heated to around 75°C and a complimentary strand of DNA to the DNA template is synthesized by the DNA polymerase using the dNTPs. This whole process is then repeated numerous times so that with each cycle the segment of DNA is replicated exponentially. As a result, the PCR product eventually contains millions of segments of the region of interest. This is then used to genotype individuals to determine which alleles of a particular gene the individual carries.

2.9.3. Agarose gel electrophoresis

Genotyping involves measuring the size of the alleles at the region of interest. There are several methods to do this, one of which is agarose gel electrophoresis. In this process, the PCR products are mixed with a coloured dye and placed in a gel made from agar and water and stained with ethidium bromide (a chemical which fluoresces under UV light in the presence of DNA). A DNA ladder (a molecule which contains fragments of known size) is also placed into the gel alongside the PCR products so that the length of the alleles of the samples can later be determined. The gel is then placed in a tank containing a buffer solution and subjected to an electric current. As the PCR products are negatively charged, the samples will move through the gel towards to the positively charged end of the tank. During this process the PCR products separate. Shorter fragments travel faster and further through the gel than longer fragments as they are smaller and can fit through pores in the agarose more easily. The gel is then placed under UV light and photographed in order to visualise the DNA bands. The bands are measured by observing how far the fragments have migrated through the gel and measuring their position relative to the DNA ladder. In each column, if only one band is

present this is an indication that the individual carries two alleles of the same length, and is thus homozygous for one of the two possible alleles (i.e. short vs. long) for the gene of interest. If two bands are present, the individual carries one of each of the possible alleles for the gene of interest and is thus heterozygous. **Figure 2.3** displays the DNA bands present for a selection of samples genotyped for *PER3*.

Figure 2.3. Example *PER3* gel electrophoresis picture



Note. On the left is the DNA ladder (HAEIII) with the base pair lengths indicated. The genotypes 1-3 are as follows: (1) homozygosity for the 4-repeat allele; (2) heterozygosity for the 4- and 5-repeat alleles; and (3) homozygosity for the 5- repeat allele.

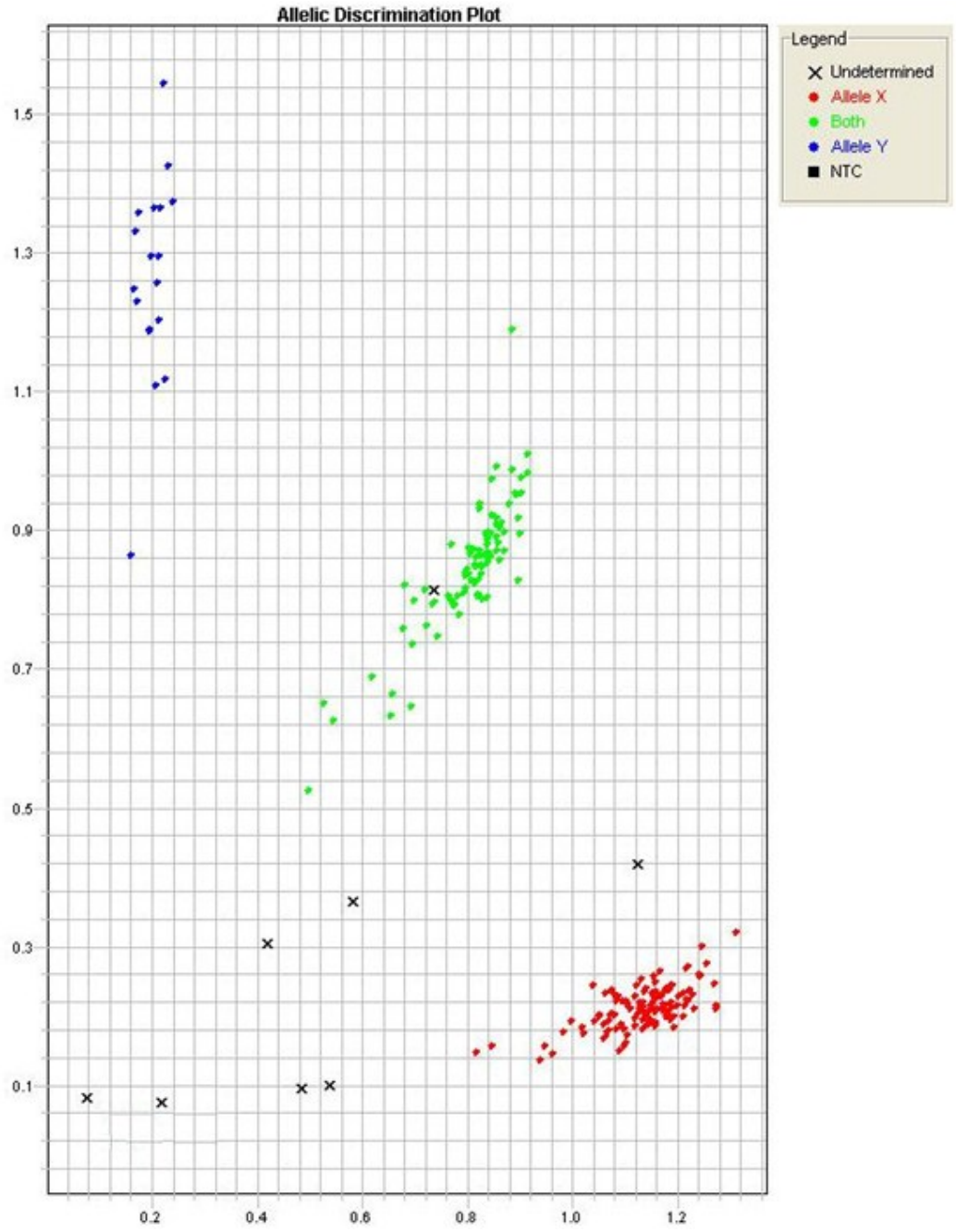
2.9.4. Taqman allelic discrimination

An alternative method to visualise the lengths of DNA fragments is Taqman® sequencing. In this method, 2 Taqman® oligonucleotide probes, which are labelled with reporter dyes (flurophores) to make them fluoresce, are included in the PCR mixture. These bind to the DNA template, and with each cycle of PCR the fluorescence of the probes is intensified. After PCR, the PCR products are placed into an Applied

Biosystems 7900HT Fast Real-Time PCR machine for allelic discrimination using the 'Sequence Detection System 2.0 (SDS)' software (Applied Biosystems). The machine scans the samples and the fluorescence emissions from the dye are collected by a laser. The relative contribution of florescence from each sample is analysed and plotted on a graph to discriminate the alleles. Low signals from the amplified reaction indicate that the fluorescence is that of the unreacted probe. An increase in signal from either one of the probes indicates homozygosity for the specific allele attached to the probe. A simultaneous increase in signal from both probes indicates heterozygosity (McGuigan & Ralston, 2002). An example of the plot used to quantify the signals, and thus determine the genotypes of the samples, is presented in **Figure 2.4**.

The decision as to whether to visualise the genotypes by agarose gel electrophoresis or by the Taqman® SDS depends on the type of polymorphism under investigation. Gel electrophoresis is used predominantly to distinguish sizes of microsatellites (short tandem repeats consisting of 2-5 nucleotides), as it is possible to distinguish between the length of varying sequences (for example, whether a particular nucleotide sequence is repeated 4 (short) or 5 (long) times). The Taqman® SDS is used to identify SNPs. However, it is also possible to identify SNPs using gel electrophoresis, and this method would be chosen if the marker under investigation consisted of a SNP within a microsatellite (as in the case of identifying the A/G SNP within the transporter region of the 5HTT- gene, described in Chapter 7).

Figure 2.4. Example graph plotted by the Taqman Sequence Detection System for allelic discrimination



Note. Along the X and Y axis is the fluorescence intensity for the 2 probes and hence the 2 alleles. The green circles in the central area of the graph show an increase in signal intensity from both probes indicating heterozygosity. The red and navy circles show an increase in intensity for only one each of the probes, indicating homozygosity for one allele accordingly. The crosses indicate samples that could not be detected.

2.10 Sample Information

The participants in this thesis come from wave 4 of the G1219 and G1219Twins longitudinal studies. G1219 initially comprised adolescent offspring of adults from a large-scale population-based study (GENESiS) (Sham, et al., 2000). Approximately 9,000 families were contacted and asked to take part in either G1219 or another study of hyperactivity in younger children, of whom a total of 3,600 families (40%) responded to at least one of the invitations (see Eley, Liang, et al., 2004 for more details). The G1219Twins are a random selection of live twin births born between 1985 and 1988 identified by the UK Office of National Statistics. Health Authorities and General Practitioners then contacted families (N = 4,000), of whom 2,947 families received the packs (Lau, Rijsdijk, & Eley, 2006), and 1,381 twin pairs responded (47% of the sample that we know received the information, 35% of the entire original sample). At wave 1 of data collection (which took place between 1999 and 2002) 3,640 respondents aged between 12 and 19 years participated in the study. Informed consent was obtained from parents/ guardians of all adolescents under 16 years, and from the adolescents themselves when over 16. Ethical approval for different stages of this study has been provided by the Research Ethics Committees of the Institute of Psychiatry, South London and Maudsley NHS Trust, and Goldsmiths, University of London. At Wave 2, data were available from 2,646 individuals (73% of the original sample at Wave 1) whilst corresponding figures for Wave 3 were 1,777 adolescents (49% of the original sample at Wave 1).

At wave 4 (which took place in 2007 and is the focus of this thesis as wave 4 was the first wave to include information about sleep) participants who had taken part in wave 2/ wave 3 were traced primarily by using websites dedicated to providing information (e.g. phone numbers and postal addresses) about members of the population. We successfully traced 2,550 individuals and sent them a questionnaire

booklet. Three reminders were then sent (a duplicate questionnaire was sent out with the last reminder in case the former had been misplaced). Participants were also emailed and telephoned in order to see whether they planned to take part. A total of 1,556 individuals were included in the wave 4 dataset (61% of those targeted; 74% of those participating at wave 3).

Zygosity was established through a questionnaire measure completed by mothers at waves 2 and 3, assessing physical similarity between twins (Cohen, Dibble, Grawe, & Pollin, 1975). When zygosity was only available from one or other wave, this rating was used. If there was disagreement between zygosity rating at the two waves, DNA was obtained (N = 26 pairs) before final classifications were made. Questionnaire methods have been shown to have around 95% accuracy in correctly determining zygosity when validated against DNA markers (Price, et al., 2000).

At wave 4, 97.6% of the sample was classified as White British, 61.5% of the sample was female, and the mode age was 20 years (range 18-27 years). Following the study design the majority of participants were close in age (90% of participants were aged 18-22 years), but the inclusion of siblings inevitably created some age-spread. At wave 4 the 1,556 individuals came from 896 families: 75 MZ male (65 complete) pairs, 76 DZ male (53 complete) pairs, 155 MZ female (125 complete) pairs, 138 DZ female (111 complete) pairs, 232 DZ opposite sex (163 complete) pairs, 44 male-male sibling (28 complete) pairs, 68 female-female sibling (44 complete) pairs, 89 opposite sex sibling (56 complete) pairs. Sibling type was uncertain for a remaining 19 (15 complete) pairs.

In the whole G1219 sample, levels of parental education were somewhat higher (39% educated to A-level or above) than in a large nationally represented sample of parents (Meltzer, Gatward, Goodman, & Ford, 2000) where 32% were educated to A-level or above. G1219 parents were also somewhat more likely to own their own houses

(82%) than in the nationally representative sample (68%). To reduce the impact of any initial response bias associated with educational level, the sample was re-weighted to match the distribution of educational qualifications in a nationally representative sample of parents (Meltzer et al., 2000). Effectively, weighting involves assigning lower weights to individuals from over-represented categories and higher weights to individuals from under-represented categories in the sample relative to the population distribution. The weights were created to be family-general, such that in model-fitting analyses, the weights did not incur any additional individual-specific effects between members of the same family. Additionally, the weight also corrected for the effects of additional attrition between waves 1 and 4. To create the response weight, predictors of attrition from each wave were first examined. The significant predictors of response were sex (girls were more likely to respond), housing tenure (response was more likely from families who owned their own houses), and from families with better educated mothers. Participating families were then weighted according to the inverse of the predicted probability of participation based on these predictor variables. The response rate was multiplied by the sampling weight and used in all analyses.

Chapter 3: Genetic and Environmental Influences on Different Components of Sleep Quality and their Overlap

3.1 Overview

This chapter investigates the extent to which genetic and environmental factors influence global sleep quality as measured by the Pittsburgh Sleep Quality Index (PSQI: Buysse, et al., 1989), as well as the individual components of sleep quality encompassed by this measure; the degree to which these components co-occur; and genetic and environmental influences on this co-occurrence. Additive genetic influences accounted for 40%$_{[.29-.50]}$, and non-shared environmental influences accounted for 60%$_{[.50-.71]}$, of the variance in global sleep quality. Genetic influence on the individual components ranged from 0-50%. The remaining source of variance was the non-shared environment, except for 'sleep duration' for which shared environmental influences were important. Phenotypic correlations between components ranged from r =.22-.59. Bivariate analyses indicated that there was substantial overlap in the genes influencing the individual components (9 of 15 correlations were ≥.50), and in general, genetic influence accounted for roughly half of the associations between components (over 40% in 11 of 15 correlations). Non-shared environmental influences were in general less correlated across the components (11 of 15 correlations were ≤.40), but owing to their greater influence on each variable still accounted for roughly half of each association (over 40% in 13 of 15 correlations). These results suggest that genetic and non-shared environmental factors are most important in explaining individual differences with regards to different components of sleep quality, although shared environment may influence sleep duration. The pattern of overlap in the genetic and environmental influences accounting for the associations between components of sleep quality is

consistent with that seen in other areas of developmental psychopathology of general genes and specific non-shared environmental influences.

3.2 Introduction

The term 'sleep quality' often refers to a collection of measurements, encompassing sleep onset latency, sleep duration, sleep efficiency, and number of awakenings, amongst other things. Although sleep quality can be inferred using objective measures to record sleep, such as by using measurements of sleep timing, duration, depth and number of sleep stage transitions using polysomnography, and from indices of rest-activity patterns using actigraphy (Buysse, et al., 2006), sleep quality can sufficiently be assessed by self-report. Subjective sleep quality has most widely been assessed, in both the general population and in clinical populations, using the PSQI (Buysse, et al., 2006). Given the reliance on this measure as an indicator of sleep quality in the field of sleep research, the more we know about the construct it claims to measure, the better. The PSQI (Buysse, et al., 1989) is a comprehensive self-report questionnaire assessing sleep disturbances in the past month, that derives ordinal scores for seven clinically relevant domains (components) of sleep: subjective sleep quality, sleep latency (the time in minutes taken to fall asleep), sleep duration, habitual sleep efficiency (a percentage score derived from sleep duration divided by the total time spent in bed), sleep disturbances (such as awakenings from sleep due to poor comfort, bad dreams etc.), use of sleeping medication and daytime dysfunction (feeling sleepy during the day as a result of a poor night's sleep). Scores from these separate components are combined to derive a global measure of sleep quality. The global score from the PSQI is typically used in many research contexts to assess sleep quality. However, it is possible that when assessed separately, or combined differently, the individual components measure qualitatively distinct aspects of sleep. Indeed, research

has demonstrated that certain components overlap to a greater extent than others and cluster together to form three distinct factors, tapping into 'sleep efficiency' (including the components 'sleep duration' and 'habitual sleep efficiency'), 'perceived sleep quality' (including 'subjective sleep quality', 'sleep latency' and 'use of sleeping medication'), and 'daily disturbances' (including 'sleep disturbances' and 'daytime dysfunction') (Cole, et al., 2006). Using a different measure to assess sleep, de Castro (2002) also found that certain components co-occur to varying degrees, with only small correlations between sleep duration and number of awakenings, and no association between sleep duration and sleep latency. Similar findings in relation to the overlap between certain sleep difficulties have been demonstrated in school aged children (Gregory, Rijsdijk, & Eley, 2006). However, it is currently unclear why certain components cluster together more strongly than others. It is possible that genetic factors, in part, account for the differing associations between components.

Previous twin research, using different measures, has highlighted the importance of genes to several of these components of sleep quality. For example, the influence of genetic factors on sleep quality, sleep length and sleep disturbance has been found to be around 33% to 44% (Heath, et al., 1990; Partinen, et al., 1983). These studies used one item to determine sleep quality. Knowledge as to the contribution of genes and environments to global sleep quality assessed by the PSQI, as well as the individual components encompassing this measure, however, is currently unknown. An examination of the relative contribution of genetic and environmental influences on the individual components of sleep is beneficial since it adds to this growing body of literature on the determinants of sleep quality, and provides a more thorough understanding of the specific constructs encompassed by the PSQI.

Genes are also known to play a role in the co-occurrence between different sleep problems (Gregory, 2008; Hublin, et al., 2001), and it is thus possible that genes

contribute to the differing associations observed between the components of sleep quality measured by the PSQI. However, the extent to which genetic and environmental influences account for the apparent associations between components is unclear and requires exploration. Bivariate analyses enable us to infer whether these aetiological influences are shared between the components, or whether they are distinct - from which inferences about their similarity could be made. Indeed, as mentioned previously (see Chapter 1, section 1.7) de Castro (2002) investigated several indices of sleep-wake behaviour and found that the genetic influences on them were largely unique. It is possible that genetic factors contribute to the greater similarity in stronger as compared to weaker associations between components. For example, genes may be more significant in explaining the association between 'subjective sleep quality' and 'sleep latency' (components which have been shown to cluster strongly), as compared to 'sleep disturbances' and 'sleep duration' (components which cluster together less strongly). Examining how genetic and environmental influences map onto the clusters described above, as well as others, will be informative in terms of gaining an understanding of the degree to which these components measure an underlying construct of sleep quality, or whether the individual components measure qualitatively distinct sleep phenotypes. Substantial genetic overlap between components would support the view that these individual components of sleep quality are conceptually similar traits, and would provide justification for measuring sleep quality using the global score. Moreover, as mentioned in Chapter 1 (section 1.8) finding genetic overlap between aspects of sleep may be useful in identifying individuals at risk for the development of sleep problems, since identifying an individual with problems in one aspect of sleep may suggest that they are genetically sensitive to correlated symptoms. Identifying specific genes involved in sleep disturbances could also be facilitated by knowledge of the overlap between specific components of sleep quality. However,

dissimilarity in the aetiological associations between components would suggest that different biological/environmental mechanisms are at play, and that the components to some extent measure widely different indices of sleep. If this is true, future research should perhaps consider the possibility that the components of sleep should be examined independently.

Accordingly, this chapter addresses the first set of research questions of this thesis, and examines: (i) the extent to which genetic and environmental factors influence global sleep quality assessed by the PSQI as well as the individual components of sleep quality encompassed by this measure; (ii) the strength of the associations between the components of sleep quality measured by the PSQI, with a focus on the clusters identified in previous research; (iii) whether there is overlap in the genetic and environmental influences on each of the components of sleep quality assessed separately; and (iv) the extent to which genetic and environmental influences account for the associations between components of sleep quality.

3.3 Method

3.3.1 Participants

The participants for this study were from the G1219 sample, and data was used from wave 4, as outlined in Chapter 2 (section 2.10).

3.3.2 Measures

Subjective sleep quality was assessed using the PSQI (Buysse, et al., 1989). The PSQI contains 18 scored items (and an additional item relating to a bed partner which is not used in the calculation of the global score). Items include both open-ended questions and fixed-choice questions. The individual 18 items encompassed by the PSQI, and the coding methods for each item, are outlined in **Table 3.1**. Using different algorithms,

items are combined to form the 7 separate scales or 'components' of sleep: subjective sleep quality; sleep latency; sleep duration; habitual sleep efficiency; sleep disturbances; use of sleeping medication; and daytime dysfunction. The components are represented as ordinal variables coded from 0-3, where 0 indicates 'no difficulty', and 3, 'severe difficulty'. The scores of different components can be summed to yield a global score with scores ranging from 0 to 21. Higher scores indicate poorer sleep quality. A score > 5 indicates a clinically significant sleep problem, and using this cut-off yields around 90% diagnostic sensitivity at distinguishing 'good' from 'poor' sleepers (Buysse, et al., 1989) (however this cut-off is not used in these analyses). The PSQI global score has previously demonstrated good psychometric properties, with both internal consistency and test-retest reliability in the .8 range (Backhaus, Junghanns, Broocks, Riemann, & Hohagen, 2002; Buysse, et al., 1989), and has favourable convergent validity when compared to other self-report sleep measures (Backhaus, et al., 2002; Carpenter & Andrykowski, 1998). In the present sample the PSQI global score yielded satisfactory internal reliability (Cronbach's alpha (*a*) = .71).

Table 3.1. Items included in Pittsburgh Sleep Quality Index

1. During the past month, when have you usually gone to bed at night?
2. During the past month, how long (in minutes) has it usually take you to fall asleep each night?
3. During the past month, when have you usually gotten up in the morning?
4. During the past month, how many hours of actual sleep did you get at night? (this may be different than the number of hours you spend in bed)

During the past month, how often have you had trouble sleeping because you...

5. Cannot get to sleep within 30 minutes

Table 3.1 (continued). Items included in Pittsburgh Sleep Quality Index

6. Wake up in the middle of the night or early morning
7. Have to get up to use the bathroom
8. Cannot breathe comfortably
9. Cough or snore loudly
10. Feel too cold
11. Feel too hot
12. Had bad dreams
13. Have pain
14. Other reason(s), please describe
15. During the past month, how would you rate your sleep quality overall?
16. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?
17. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?
18. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

Note. Items 1-4 participants are required to indicate specific times/hours/minutes; Items 5-14, 16 and 17 are coded as 0 = not during the past month; 1 = less than once a week; 2 = once or twice a week; 3 = three or more times a week; Item 15 is coded as 0 = very good; 1 = fairly good; 2 = fairly bad; 3 = very bad; Item 18 is coded as 0 = no problem at all; 1 = only a very slight problem; 2 = somewhat of a problem; 3 = a very big problem.

3.3.3 Statistical analysis

Descriptive statistics were performed using the Statistical Package for the Social Sciences (SPSS, 2001). Intraclass correlations (to assess global sleep quality), polychoric correlations (to assess the categorical sleep components) and genetic model

fitting analyses were carried out using Mx (Neale, 1997), as described in section 2.6.1, and incorporated the weight variable described in section 2.10 to account for selection bias and attrition.

3.3.3.1 Data preparation

3.3.3.1.1 Age and sex regression

Prior to analysis, the PSQI data were regressed for the effects of age and sex. Because twins within a pair share a common age and sex (in MZ and DZ same-sex pairs) similarity on a trait of interest may be partially due to these factors. In standard twin designs assessing quantitative traits it is necessary to correct for these age-sex effects as failure to do so can result in overestimation of the twin intraclass correlations (McGue & Bouchard, 1984). Age-sex regression is achieved by partialling out the effects of age and sex on the variable of interest using linear regression, and using the resulting unstandardised residual as the dependent variable (sleep quality). This technique is standard in twin model-fitting analyses of quantitative traits.

3.3.3.1.2 Categorical variable coding

The 7 components were originally coded as 4 category ordinal variables (coded 0-3). However, in the current sample, examination of the frequency distribution of scores indicated that few individuals (ranging from 1.3% to 13.3% of the sample) scored at the upper extreme (a score of 3) for the components. Categories of scores 2 and 3 were therefore collapsed to yield 3 discrete categories ranging from 0-2, where 0 indicated ‘no difficulty’, 1 = ‘mild difficulty’ and 2 = ‘moderate/severe difficulty’.

3.3.3.2 Univariate modelling of global sleep quality

In order to assess the extent to which genetic and environmental factors influence global sleep quality assessed as a quantitative trait, standard univariate genetic analyses as outlined in Chapter 2 (section 2.4) were carried out.

3.3.3.3 Liability threshold modelling

It is not possible to model categorical data in genetically sensitive designs in the same way as quantitative measures using the methods of quantitative genetic analysis outlined in Chapter 2. An approach to modelling categorical data in genetically sensitive designs is to use a liability threshold model. Liability threshold modelling is based on the assumption that the ordered categories of a variable have an underlying normal distribution, and that this liability distribution has threshold values discriminating the categories (Neale & Cardon, 1992). In other words, this model assumes that the contribution of all of the genetic and environmental influences on the liability of a trait sum to an underlying normally distributed liability continuum. In the context of a single variable, the thresholds are estimated so that the exact proportion of the distribution between thresholds reflects the observed proportions of the sample falling into each category. Thus, when a certain threshold of liability is reached, the individual falls into the corresponding category. In the present analyses, 2 thresholds were specified for each sleep component variable to model the three categories of symptoms, 'no difficulty', 'mild difficulty' and 'moderate/severe difficulty'. Using the proportions of the sample falling into each category for each variable, threshold values were calculated as the critical z-values (assuming a normal distribution with mean of 0 and standard deviation [SD] of 1) on the z-distribution which partition the distribution according to the cell frequencies. The first threshold represents the proportion of the sample scoring 0, and so is calculated as the z-value representing the corresponding proportion. The second

threshold represents the *increment* between the first z-value and a second z-value, with the second z-value placed according to the proportion of cases in the highest category, e.g. scoring 2. The critical z-values and threshold values for each sleep component variable are presented in **Table 3.2**.

Table 3.2. Critical z-values estimated from relative cell proportions of data in each category for the sleep components

Sleep Component	Lower z-value (1st threshold)	Upper z-value	Increment (2nd threshold)
1. Subjective Sleep Quality	-.93	.70	1.63
2. Sleep latency	-.83	.42	1.25
3. Sleep Duration	.19	1.61	1.42
4. Habitual Sleep Efficiency	.40	1.20	.80
5. Sleep Disturbances	-1.68	.71	2.39
6. Use of Sleeping Medication	1.44	2.1	.66
7. Daytime Dysfunction	-.72	.96	1.68

Note. The upper z-value is subtracted from the lower z-value to give the incremental z-score (the 2nd threshold value). For example, the relative proportions in the categories 0, 1 and 2 for subjective sleep quality, were 17.6%, 57.1% and 24.3%, respectively. The z-value partitioning the normal distribution at 17.6% is -.93, and the z-value partitioning the upper 24.3% is .70. Thus, the difference between these values is 1.63 (the polarity of the value is reversed so that the threshold marks the upper tail of the distribution).

In the bivariate case, it is assumed that the joint distribution of such liabilities have a multivariate normal distribution from which correlations and thresholds are estimated from the relative cell proportions of the data (Neale & Cardon, 1992). Thus, in the bivariate case, for each combination of any two sleep component variables there would be a contingency table with 9 cells of data, representing the 3 categories for each variable.

3.3.3.4 Polychoric correlations

Prior to genetic model fitting, polychoric correlations (rather than intraclass correlations) between the underlying liabilities for the traits were estimated by model fitting of the MZ, DZ and sibling data. Polychoric correlations estimate the relationship between two ordinal variables, assuming that their underlying distributions reflect the normal distribution. In univariate analyses (e.g. subjective sleep $quality_{twin1}$ and subjective sleep $quality_{twin2}$), the cross-twin/sibling same-trait correlations are estimated in pairs of MZ twins, DZ twins and non-twin siblings separately. The difference in similarity between these groups is used to estimate genetic and environmental influences upon traits as modelled in the univariate genetic analyses.

To assess the heritability and overlap between the individual components of sleep quality, a series of bivariate models were analysed, assessing the phenotypic correlations and relative contribution of A, C, and E; and A, D and E, to every combination of any two components. Although a multivariate model containing all variables simultaneously would have been favourable over conducting numerous bivariate models, this was not run because of the computationally intensive integration method used. In bivariate analyses, to simplify the interpretation of the data, the correlations (measured in pairs of MZ/DZ/sibling pairs separately) are constrained to provide: one within-twin/sibling cross-trait correlation (e.g. subjective sleep $quality_{twin1}$ and sleep $latency_{twin1}$: equated between MZ/DZ/sibling groups, as these overall estimates would not be expected to differ by zygosity); three cross-twin/sibling same-trait correlations (e.g. subjective sleep $quality_{twin1}$ and subjective sleep $quality_{twin2}$: one each for MZ/DZ/sibling group separately, as the dissimilarity between zygosity groups will give an indication of heritability); and three cross-twin/sibling cross-trait correlations (e.g. subjective sleep $quality_{twin1}$ and sleep $latency_{twin2}$: one each for MZ/DZ/sibling group separately, as the dissimilarity between zygosity groups will give

an indication of bivariate heritability). The interpretation of the differences in magnitude of these correlations is outlined in Chapter 2 (section 2.7).

3.3.3.5 Genetic model fitting

Maximum-likelihood genetic model fitting estimates the model parameters (A, C, D and E) from the observed raw MZ, DZ and sibling data. Model fitting uses the differences in MZ and DZ twin/sibling correlations, and rests on the assumption that the variance of the liabilities is the sum of the contribution of genetic and environmental influences. In the bivariate analyses, the aim is to examine the extent to which the correlation between two traits is due to genetic or environmental overlap.

For the univariate analysis of global sleep quality as well as the threshold analyses of the categorical data, models including additive genetic (A), shared environmental (C) *or* non-additive genetic (D), and non-shared environmental (E) variance components were examined. As mentioned in Chapter 2 (section 2.4) it is not possible to model both shared environmental effects and non-additive genetic effects simultaneously as they predict different MZ and DZ twin correlation ratios. These effects are examined in separate models, and the best fitting model is selected for interpretation. Furthermore, it is possible to examine nested models, where certain parameters (e.g. C) are dropped from the model by fixing the parameters to zero, to determine whether their exclusion results in a significant worsening of fit. Parameters which did not result in a significant worsening of fit when dropped were excluded from the models in order to adhere to parsimony. See Chapter 2 (section 2.6.2) for an overview of the model fitting procedures.

3.3.3.6 Sex differences

Differences between males in females in terms of their overall PSQI score, as well as sex differences in the prevalence of the individual PSQI components were tested. In addition, in the genetic analyses, quantitative, qualitative and scalar sex differences were tested in the univariate genetic analysis of global sleep quality (for further details of these concepts see Chapter 2, section 2.8). In the threshold analyses of the individual components, however, only quantitative sex differences were explored (this was because there was no evidence for sex differences of any type in the investigation of global sleep quality and so further investigation of this was not considered warranted. An investigation of quantitative sex differences was undertaken, however, to further confirm the lack of effect). Quantitative sex differences were explored in the bivariate analyses as is standard practice in analyses of this kind.

3.4 Results

3.4.1 Descriptive statistics

The frequencies of scores on the global PSQI are displayed in **Figure 3.1**. Skew was not considered problematic for the global PSQI score (skew = .98, [SE = .09]), and so this variable was not transformed for this purpose. **Table 3.3** displays means and standard deviations of raw scores for global sleep quality split by sex and zygosity. There were no significant differences between the sexes on sleep quality (change in fit of a model where means and standard deviations between males and females are free to vary, compared to a model where this information is equated between the sexes: $\Delta\chi^2$ = 0.65, $\Delta df = 2$, p=.72).

Figure 3.1. Histogram of the frequency of global PSQI scores

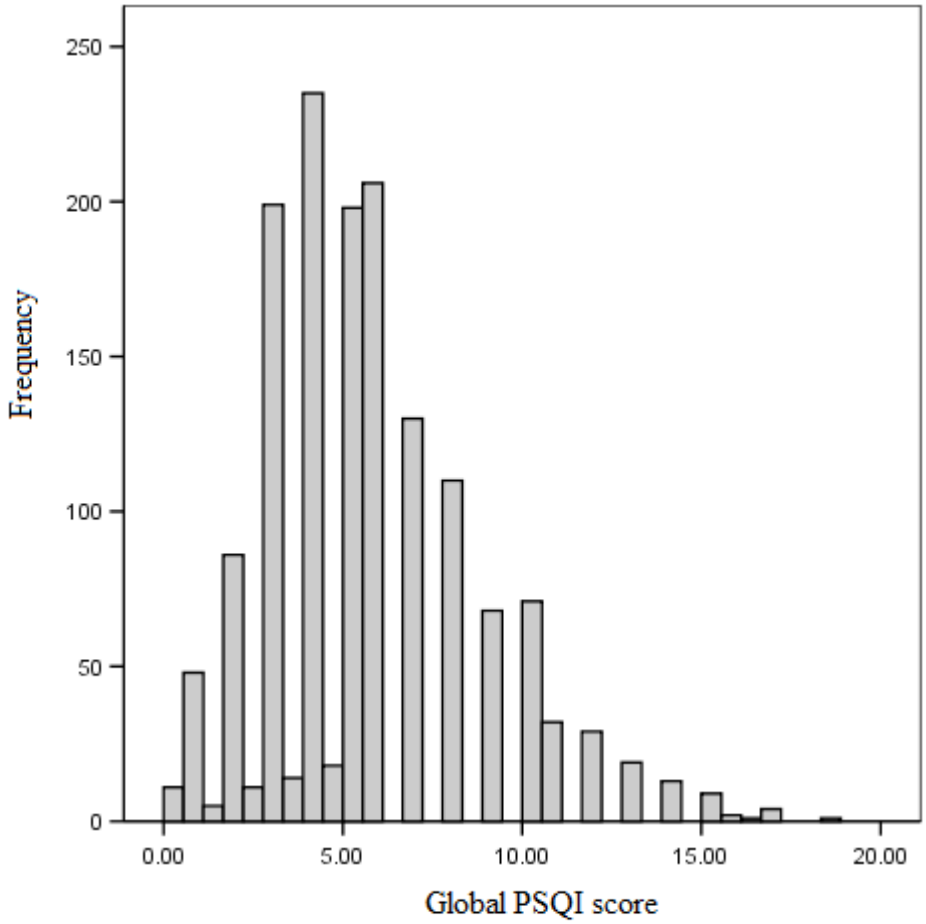


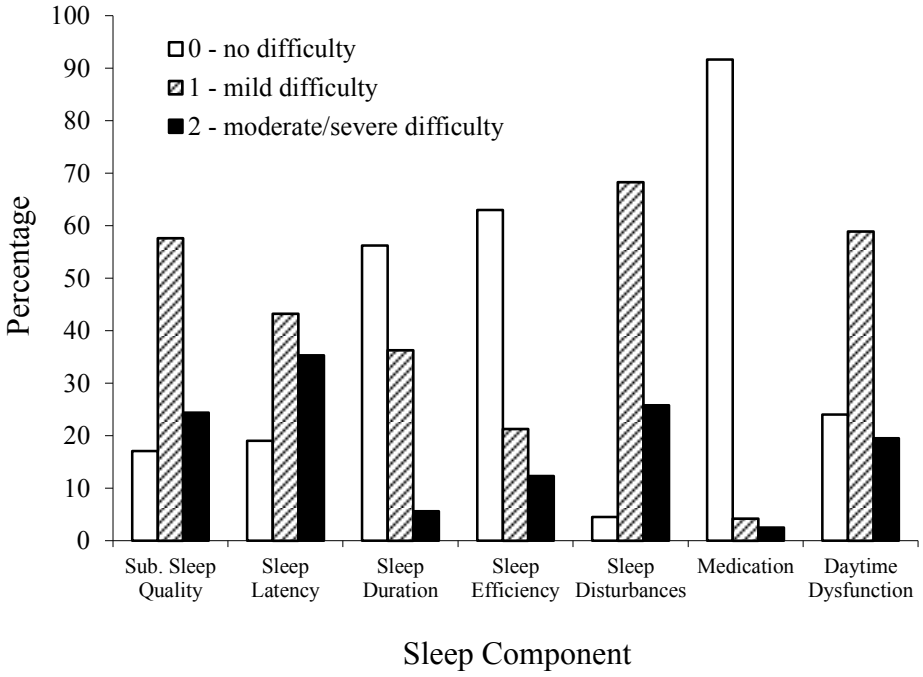
Table 3.3. Descriptive statistics. Means (standard deviations) of scores for global sleep quality

	Total	Males	Females	MZ	DZ	Sibs
PSQI	5.66 (3.01)	5.58 (3.00)	5.72 (3.01)	5.45 (2.86)	5.74 (3.10)	5.70 (2.93)

Note. PSQI = Pittsburgh Sleep Quality Index (range = 0-21); Means and standard deviations of raw (untransformed) data. Sex differences for means and standard deviations were tested, * p <.01.

The frequency of responses, which ranged from 0 (no difficulty) to 2 (moderate/severe difficulty), for the 7 components of sleep quality is presented in **Figure 3.2**. As expected, different types of sleep phenotypes had widely differing prevalences. For 'sleep quality', 'sleep latency', 'sleep disturbances', and 'daytime dysfunction', the majority of the sample reported having mild difficulties rather than none or moderate/severe difficulties. For 'sleep duration', 'habitual sleep efficiency' and 'use of sleeping medication', however, the majority of the sample reported no difficulties, and fewer than 40% reported mild difficulties. As 'use of sleeping medication' was reported as 'no difficulty' (i.e. participants were not using sleeping medications) for the majority of the sample (91.6%), this component was not decomposed into genetic and environmental influences as the frequencies in the other categories of responses to this variable were too small to be included in further analysis.

Figure 3.2. Percentage of cases scoring 0, 1, or 2 for each component of the PSQI



Note. Figure taken from Barclay et al. (2010).

There were significant sex differences in the frequencies of cases falling into the severity categories for sleep disturbances, $\chi^2(2) = 33.98$, $p<.001$. A significantly higher proportion of females (31%) reported moderate/severe difficulties in sleep disturbances than males (19%). There were no significant differences between males and females in the frequencies of cases falling into each of the severity categories for the other sleep components.

3.4.2 Phenotypic correlations

Phenotypic correlations between all components (excluding 'use of sleeping medication' which was excluded because the majority of participants were not using sleeping medications) are presented in **Table 3.4**. The phenotypic correlations between all components ranged from r = .22-.59, the weakest association between 'habitual sleep efficiency' and 'daytime dysfunction' and the strongest between 'sleep duration' and 'habitual sleep efficiency' (which includes sleep duration in its calculation). For the three specific clusters outlined by Cole and colleagues (2006) the phenotypic correlations were moderate: 'subjective sleep quality' and 'sleep latency' (r = .58); 'sleep duration' and 'habitual sleep efficiency' (r = .59); and 'sleep disturbances' and 'daytime dysfunction' (r = .42). Confidence intervals (95%) excluded zero, indicating that all phenotypic correlations were significant.

Table 3.4. Phenotypic correlations (with 95% confidence intervals) between sleep components

Sleep Component	1. Subjective Sleep Quality	2. Sleep Latency	3. Sleep Duration	4.Habitual Sleep Efficiency	5. Sleep Disturbances	7. Daytime Dysfunction
1. Subjective Sleep Quality	1					
2. Sleep Latency	.58 (.53 - .63)	1				
3. Sleep Duration	.47 (.41 - .53)	.31 (.24 - .37)	1			
4. Habitual Sleep Efficiency	.47 (.39 - .52)	.45 (.39 - .51)	.59 (.53 - .65)	1		
5. Sleep Disturbances	.48 (.41 - .54)	.47 (.41 - .53)	.23 (.15 - .30)	.33 (.25 - .41)	1	
7. Daytime Dysfunction	.43 (.37 - .49)	.33 (.26 - .39)	.26 (.19 - .33)	.22 (.14 - .29)	.42 (.35 - .48)	1

Note. All phenotypic correlations were obtained from Mx incorporating a weight to account for selection bias and attrition. Component 6 (use of sleeping medication) was excluded from all analyses due to the low frequencies in some categories of scores.

3.4.3 Twin/sibling correlations

The cross-twin/sibling within trait correlations are presented in **Table 3.5**. All univariate twin correlations (except for sleep latency and sleep duration) were greater for MZ than DZ twins or siblings, (ranging from .22-.52 for MZ, and .03-.28 for DZ twins/siblings) suggesting genetic influence on global sleep quality as well as the individual components of sleep quality. For 'global sleep quality', 'subjective sleep quality', 'habitual sleep efficiency', and 'daytime dysfunction' the MZ correlations were greater than double the DZ/sibling correlations suggesting that non-additive genetic factors may be important for these phenotypes. For 'sleep latency' the MZ twin correlations were of similar magnitude to the sibling correlations (MZ = .22; siblings =

.24). Furthermore, the twin/sibling correlations for 'sleep duration' were of similar magnitude between all pairs (MZ = .23; DZ = .28; siblings = .26) suggesting that genetic influence was not important and that shared environmental influences accounted for twin/sibling similarity.

Table 3.5. MZ, DZ and sibling cross-twin/sibling within trait correlations (with 95% confidence intervals) for sleep components

Sleep Component	Global Sleep Quality	1.Subjective Sleep Quality	2. Sleep Latency	3. Sleep Duration	4. Habitual Sleep Efficiency	5. Sleep Disturbances	7. Daytime Dysfunction
MZ Twins	.41 (.28 - .52)	.49 (.31 - .63)	.22 (.03 - .40)	.23 (.02 - .42)	.32 (.09 - .51)	.41 (.24 - .57)	.52 (.37 - .65)
DZ Twins	.19 (.08 - .30)	.13 (.00 - .26)	.08 (-.06 - .21)	.28 (.13 - .41)	.17 (.01 - .31)	.21 (.03 - .37)	.16 (.02 - .30)
Siblings	.13 (-.05 - .31)	.16 (-.08 - .38)	.24 (.00 - .45)	.26 (-.01 - .49)	.03 (-.27 - .33)	.04 (-.24 - .31)	.13 (-.11 - .35)

Note. MZ = monozygotic; DZ = dizygotic. All twin correlations were obtained from Mx incorporating a weight to account for selection bias and attrition. The model was constrained where appropriate. For example, the twin correlations were constrained so that those of the randomly selected twin 1's were the same as the randomly selected twin 2's. Component 6 (use of sleeping medication) was excluded from all analyses due to the low frequencies in some categories of scores.

Bivariate cross-twin/sibling cross-trait correlations (e.g. 'sleep latency'$_{twin\ 1}$ and 'sleep duration'$_{twin\ 2}$) are presented in **Table 3.6** for MZ/DZ twins and **Table 3.7** for siblings. For most pairs of variables (with the exception of 'sleep duration and habitual sleep efficiency'; and 'habitual sleep efficiency and daytime dysfunction') cross-twin/sibling cross-trait correlations were greater for MZ twins (ranging from .05-.33) than DZ twins/siblings (ranging from -.03-.23), suggesting genetic influence on these

associations. For 5 of these associations, MZ twin correlations were more than double the DZ/sibling cross-twin/sibling cross-trait correlations, suggesting that non-additive genetic factors may be important.

Table 3.6. MZ and DZ cross-twin cross-trait correlations (with 95% confidence intervals) for sleep component scores from bivariate models

Sleep Component	1. Subjective Sleep Quality	2. Sleep Latency	3. Sleep Duration	4. Habitual Sleep Efficiency	5. Sleep Disturbances	7. Daytime Dysfunction
1. Subjective Sleep Quality	/	.20 (.06 - .33)	.23 (.08 - .36)	.28 (.13 - .41)	.31 (.19 - .43)	.27 (.15 - .38)
2. Sleep Latency	.12 (.02 - .22)	/	.05 (-.10 - .20)	.18 (.02 - .32)	.21 (.07 - .34)	.18 (.06 - .30)
3. Sleep Duration	.14 (.03 - .24)	.02 (-.08 - .13)	/	.09 (-.08 - .25)	.25 (.12 - .38)	.09 (-.04 - .22)
4. Habitual Sleep Efficiency	.12 (.02 - .23)	.12 (.01 - .22)	.11 (.00 - .23)	/	.33 (.19 - .46)	.10 (-.04 - .23)
5. Sleep Disturbances	.02 (-.09 - .14)	.19 (.08 - .30)	.14 (.02 - .25)	.06 (-.06 - .18)	/	.17 (.05 - .29)
7. Daytime Dysfunction	.09 (-.02 - .19)	.09 (-.01 - .19)	.07 (-.03 - .18)	.10 (-.01 - .20)	.04 (-.07 - .16)	/

Note. Above diagonal: MZ = monozygotic twins. Below diagonal: DZ = dizygotic twins. All cross-twin cross-trait correlations were obtained from Mx incorporating a weight to account for selection bias and attrition. Component 6 (use of sleeping medication) was excluded from all analyses due to the low frequencies in some categories of scores.

Table 3.7. Sibling cross-sibling cross-trait correlations (with 95% confidence intervals) for sleep component scores from bivariate models

Sleep Component	1. Subjective Sleep Quality	2. Sleep Latency	3. Sleep Duration	4. Habitual Sleep Efficiency	5. Sleep Disturbances	7. Daytime Dysfunction
1.Subjective Sleep Quality	/					
2. Sleep Latency	.15 (-.04 - .32)	/				
3. Sleep Duration	.16 (-.03 - .34)	.07 (-.11 - .24)	/			
4. Habitual Sleep Efficiency	.10 (-.10 - .29)	.05 (-.15 - .24)	.16 (-.07 - .36)	/		
5.Sleep Disturbances	-.00 (-.20 - .19)	.15 (-.05 - .33)	-.03 (-.23 - .17)	.04 (-.17 - .26)	/	
7. Daytime Dysfunction	.08 (-.10 - .25)	.07 (-.10 - .23)	.03 (-.16 - .20)	.09 (-.11 - .28)	.23 (.04 - .42)	/

Note. All cross-sibling cross-trait correlations were obtained from Mx incorporating a weight to account for selection bias and attrition. Component 6 (use of sleeping medication) was excluded from all analyses due to the low frequencies in some categories of scores.

3.4.4 Univariate model fitting analyses

Univariate genetic model fitting analyses for global sleep quality are presented in **Table 3.8**. The table first displays the fit of the saturated (fully unconstrained) model. Subsequently, qualitative, quantitative and scalar sex differences are tested, as are the significance of C and D. An 'AE' model in which sex differences were equated was considered the best-fitting model (i.e. not fitting significantly worse than fuller models [i.e. models allowing for sex differences or where C was included]; and as indicated by the lowest, negative AIC value). Additive genetic influences accounted for $40\%_{[.29\text{-}.50]}$, and non-shared environmental influences accounted for $60\%_{[.50\text{-}.71]}$, of the variance in global sleep quality.

Table 3.8. Fit statistics for univariate genetic model fitting analyses of global sleep quality

	Model Fit		Fit relative to saturated model			
Model	-2LL	*df*	$\Delta\chi^2$	Δdf	*p*	AIC
1. Saturated	5705.55	1275				
2. ACE QSD + Qual. SD on A	5725.99	1292	20.44	17	.25	-13.56
3. ACE QSD + Qual. SD on C	5726.05	1292	20.50	17	.25	-13.50
4. ADE QSD + Qual. SD on A	5725.96	1292	20.41	17	.25	-13.59
5. ADE QSD + Qual. SD on D	5725.98	1292	20.43	17	.25	-13.57
6. ACE QSD	5726.05	1293	20.50	18	.31	-15.50
7. ADE QSD	5726.08	1293	20.53	18	.30	-15.47
8. ACE SSD	5729.86	1295	24.31	20	.23	-15.69
9. ADE SSD	5729.19	1295	23.64	20	.26	-16.36
10. AE SSD	5729.86	1296	24.31	21	.28	-17.69
11. DE SSD	5730.91	1296	25.36	21	.23	-16.64
12. ACE NSD	5729.98	1296	24.43	21	.27	-17.58
13. ADE NSD	5729.32	1296	23.77	21	.30	-18.24
***14. AE NSD**	**5729.98**	**1297**	**24.43**	**22**	**.33**	**-19.58**
15. CE NSD	5739.41	1297	33.86	22	.05	-10.14
16. DE NSD	5731.04	1297	25.49	22	.27	-18.51
17. E NSD	5774.22	1298	68.67	23	.00	22.67

Note. * = Best-fitting model; QSD = Quantitative sex differences (magnitude of parameter estimates can vary between males and females); Qual. SD = Qualitative sex differences on A, C or D (genetic or shared environmental correlation between males and females); SSD = Scalar sex differences (variance differences between males and females); NSD = no sex differences; -2LL = -2*(log likelihood); df = degrees of freedom; $\Delta\chi^2$ and Δdf = change in chi-square statistic and corresponding degrees of freedom (computed as the difference in likelihood and df between each model and the saturated model); *p* = probability; AIC – Akaike's Information Criterion statistic (calculated as $\Delta\chi^2 - 2\Delta df$). All analyses focus on transformed variables. All estimates were obtained from Mx and incorporated a weight to account for initial selection bias and selective attrition.

Univariate genetic model fitting analyses for the individual components of sleep quality are presented in **Table 3.9**. The parameter estimates presented are those from the best fitting models (those that did not fit significantly worse than saturated models and had the lowest AIC values). Shared environmental influences (C) could be dropped from all models except for 'sleep duration' without resulting in a significant worsening of fit to the data, indicating that this source of variance was not significant. Genetic influences (additive or non-additive effects accordingly) could not be dropped without significantly reducing the fit and so additive or non-additive genetic influences were retained in all models (with the exception of 'sleep duration'), indicating that genetic factors were significant for these components. There were no significant differences in the magnitude of genetic and environmental influences on the sleep components between males and females (indicated by no significant worsening in fit of models in which quantitative sex differences were equated compared to models where the parameters were free to vary between the sexes [all $\Delta\chi^2 = p > .05$]). All univariate models provided an adequate fit to the data, not fitting significantly worse than saturated models ($\Delta\chi^2 = p > .05$). Genetic influence (both additive and non-additive effects) on the components (with the exception of 'sleep duration') ranged from 23%-50%, with the remaining source of variance due to the non-shared environment (ranging from 50%-77%). For 'sleep duration', shared and non-shared environmental influences accounted for the entire variation between individuals.

Table 3.9. Fit statistics and parameter estimates from univariate genetic analyses

		Genetic model fit		Fit relative to saturated model				Parameter estimates from best fitting model		
		-2LL	*df*	$\Delta\chi^2$	Δdf	*p*	AIC	A	C/D	E
1. Subjective Sleep Quality	SAT	1673.87	1293							
	ACE	1696.62	1327	22.75	34	.93	-45.25	/	.50 (.34 - .63)	.50 (.37 - .66)
	ADE	1694.37	1327	20.5	34	.97	-47.5			
	AE	1696.62	1328	22.75	35	.94	-47.25			
	CE	1704.99	1328	31.12	35	.66	-38.88			
	***DE**	**1694.43**	**1328**	**20.56**	**35**	**.78**	**-49.44**			
	E	1724.76	1329	50.89	36	.05	-21.11			
2. Sleep Latency	SAT	1865.25	1271							
	ACE	1901.68	1305	36.43	34	.36	-31.57	.23 (.08 - .37)	/	.77 (.63 - .92)
	ADE	1901.70	1305	36.45	34	.35	-31.55			
	***AE**	**1901.70**	**1306**	**36.45**	**35**	**.40**	**-33.55**			
	CE	1902.51	1306	37.26	35	.37	-32.74			
	DE	1902.65	1306	37.40	35	.36	-32.60			
	E	1910.72	1307	45.47	36	.13	-26.53			
3. Sleep Duration	SAT	1340.28	1279							
	ACE	1358.69	1313	18.41	34	.99	-49.59	/	.26 (.15 - .37)	.74 (.63 - .85)
	ADE	1362.29	1313	22.01	34	.94	-45.99			
	AE	1362.29	1314	22.01	35	.96	-47.99			
	***CE**	**1358.69**	**1314**	**18.41**	**35**	**.99**	**-51.59**			
	DE	1368.03	1314	27.75	35	.80	-42.25			
	E	1380.17	1315	39.89	36	.30	-32.11			
4. Habitual Sleep Efficiency	SAT	1396.77	1262							
	ACE	1426.26	1296	29.49	34	.69	-38.51	.30 (.13 - .46)	/	.70 (.54 - .87)
	ADE	1426.22	1296	29.45	34	.69	-38.55			
	***AE**	**1426.26**	**1297**	**29.49**	**35**	**.73**	**-40.51**			
	CE	1428.05	1297	31.28	35	.65	-38.72			
	DE	1426.88	1297	30.11	35	.70	-39.89			
	E	1437.91	1298	41.14	36	.26	-30.86			
5. Sleep Disturbances	SAT	1034.30	1283							
	ACE	1064.49	1317	30.20	34	.65	-37.80	.39 (.23 - .53)	/	.61 (.47 - .77)
	ADE	1064.46	1317	30.17	34	.65	-37.83			
	***AE**	**1064.49**	**1318**	**30.20**	**35**	**.70**	**-39.80**			
	CE	1068.70	1318	34.41	35	.50	-35.59			
	DE	1064.85	1318	30.55	35	.68	-39.45			
	E	1087.81	1319	53.51	36	.03	-18.49			
7. Daytime Dysfunction	SAT	1605.02	1275							
	ACE	1637.86	1309	32.84	34	.52	-35.16	/	.53 (.39 - .65)	.47 (.35 - .61)
	ADE	1635.43	1309	30.41	34	.64	-37.59			
	AE	1637.86	1310	32.84	35	.57	-37.16			
	CE	1649.33	1310	44.31	35	.13	-25.69			
	***DE**	**1635.44**	**1310**	**30.42**	**35**	**.69**	**-39.58**			
	E	1676.92	1311	71.90	36	.00	-0.10			

Note. * = best fitting model; -2LL = minus twice the log-likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in -2LL between the saturated model and the genetic model; Δdf = change in degrees of freedom between the saturated model and the genetic model; *p* = probability; AIC = Akaike's information criterion (calculated as

$\Delta\chi^2 - 2\Delta df$); A = genetic influence; C = shared environmental influence; D = non-additive genetic influence; E = non-shared environmental influence. All analyses were obtained from Mx incorporating a weight to account for selection bias and attrition. Component 6 (use of sleeping medication) was excluded from all analyses due to the low frequencies in some categories of scores.

3.4.5 Bivariate model fitting analyses

Model fitting information from the bivariate genetic models is presented in **Table 3.10**. Most full bivariate model fitting analyses (with the exception of two: the association between 'sleep latency and daytime dysfunction'; and the association between 'habitual sleep efficiency and sleep disturbances') did not fit significantly worse than saturated models ($\Delta\chi^2 = p > .05$), and so provided an adequate fit to the data. Nested models, in which the influence of the shared environment or non-additive genetic influence was fixed to zero, were considered to be the models of best fit (as indicated by large, negative AIC values) compared to the full ACE or ADE models for all associations except 3 (the associations between 'sleep latency and sleep duration'; 'sleep duration and habitual sleep efficiency'; and 'habitual sleep efficiency and 'sleep disturbances), indicating that these sources of variance were not significant and could be dropped from the models.[1] For the associations between 'sleep latency and sleep duration'; 'sleep duration and habitual sleep efficiency', and 'habitual sleep efficiency and sleep disturbances', nested models in which additive genetic influence was fixed to zero were considered to be the best fitting models, indicating that this source of variance could be dropped from these models without significantly worsening their fit.

[1] Of note, 'DE' models were not tested in the bivariate analyses in order to simplify the interpretation of the numerous models, and because in terms of broad-sense heritability 'AE' models incorporate all genetic effects. 'E' models were not tested in the bivariate analyses as the univariate analyses indicated that these fit significantly poorly and so were not considered appropriate here.

Table 3.10. Fit statistics for bivariate genetic model fitting analyses

		Saturated model fit		Genetic model fit		Fit relative to saturated model			
		-2LL	*df*	-2LL	*df*	$\Delta\chi^2$	Δ *df*	*p*	AIC
1. Subjective sleep quality &	ACE	4090.03	2607	4124.34	2630	34.31	23	.06	-11.69
	ADE			4120.24	2630	30.21	23	.14	-15.79
2. Sleep latency	***AE**			**4124.34**	**2633**	**34.31**	**26**	**.13**	**-17.69**
	CE			4133.50	2633	43.47	26	.02	-8.53
1. Subjective sleep quality &	ACE	3703.20	2615	3723.91	2638	20.71	23	.60	-25.29
	ADE			3724.12	2638	20.92	23	.59	-25.08
3. Sleep duration	***AE**			**3726.97**	**2641**	**23.77**	**26**	**.59**	**-28.23**
	CE			3731.52	2641	28.32	26	.34	-23.68
1. Subjective sleep quality &	ACE	3781.97	2598	3804.44	2621	22.47	23	.49	-23.53
4. Habitual Sleep Efficiency	ADE			3801.35	2621	19.38	23	.68	-26.62
	***AE**			**3803.77**	**2624**	**21.80**	**26**	**.70**	**-30.20**
	CE			3813.77	2624	31.80	26	.20	-20.20
1. Subjective sleep quality &	ACE	3408.77	2619	3432.36	2642	25.59	23	.32	-20.41
5. Sleep disturbances	ADE			3429.05	2642	20.28	23	.62	-25.72
	***AE**			**3433.84**	**2645**	**26.07**	**26**	**.51**	**-26.93**
	CE			3444.77	2645	36.00	26	.09	-16.00
1. Subjective sleep quality &	ACE	3981.54	2611	4015.95	2634	34.41	23	.06	-11.59
7. Daytime dysfunction	ADE			4012.91	2634	31.37	23	.11	-14.63
	***AE**			**4015.94**	**2637**	**34.40**	**26**	**.12**	**-17.60**
	CE			4034.45	2637	52.91	26	.00	.91
2. Sleep latency &	ACE	3998.94	2593	4026.91	2616	27.97	23	.22	-18.03
3. Sleep duration	ADE			4030.31	2616	31.37	23	.11	-14.63
	AE			4030.93	2619	31.99	29	.19	-20.01
	***CE**			**4027.94**	**2619**	**29.00**	**29**	**.31**	**-23.00**
2. Sleep latency &	ACE	3977.92	2576	4011.68	2599	33.76	23	.07	-12.24
4. Habitual sleep efficiency	ADE			4010.11	2599	32.19	23	.10	-13.81
	***AE**			**4010.16**	**2602**	**32.24**	**26**	**.18**	**-19.76**
	CE			4012.51	2602	32.59	26	.12	-17.41
2. Sleep latency &	ACE	3611.83	2597	3637.84	2620	26.01	23	.30	-19.99
5. Sleep disturbances	ADE			3637.12	2620	25.29	23	.33	-20.71
	***AE**			**3638.24**	**2623**	**26.41**	**26**	**.44**	**-25.59**
	CE			3641.62	2623	26.79	26	.28	-22.21
2. Sleep latency &	ACE	4248.34	2589	4288.60	2612	40.26	23	.01	-5.74
7. Daytime dysfunction	ADE			4285.44	2612	37.10	23	.03	-8.90
	***AE**			**4288.22**	**2615**	**39.88**	**26**	**.04**	**-12.12**
	CE			4300.20	2615	51.86	26	.00	-0.14
3. Sleep duration &	ACE	3340.02	2584	3350.29	2607	10.27	23	.99	-35.73
4. Habitual sleep efficiency	ADE			3353.85	2607	13.83	23	.93	-32.17
	AE			3354.16	2610	14.14	26	.97	-37.86
	***CE**			**3353.18**	**2610**	**13.16**	**26**	**.98**	**-38.84**
3. Sleep duration &	ACE	3202.24	2605	3222.09	2628	19.85	23	.65	-26.15
5. Sleep disturbances	ADE			3225.43	2628	23.19	23	.45	-22.81
	***AE**			**3225.63**	**2631**	**23.39**	**26**	**.61**	**-28.61**
	CE			3228.07	2631	25.83	26	.47	-26.17

Table 3.10 (continued). Fit Statistics for Bivariate Genetic Model Fitting Analyses

		Saturated model fit		Genetic model fit		Fit relative to saturated model			
		-2LL	*df*	-2LL	*df*	$\Delta\chi^2$	Δ *df*	*p*	AIC
3. Sleep duration & 7. Daytime dysfunction	ACE	3758.21	2597	3782.80	2620	24.59	23	.38	-21.41
	ADE			3783.64	2620	25.43	23	.33	-20.57
	***AE**			**3786.36**	**2623**	**28.15**	**26**	**.35**	**-23.85**
	CE			3794.26	2623	36.05	26	.09	-15.95
4. Habitual sleep efficiency & 5. Sleep disturbances	ACE	3228.45	2588	3313.98	2611	85.53	23	.00	39.53
	ADE			3314.79	2611	86.34	23	.00	40.34
	AE			3316.22	2614	87.77	26	.00	35.77
	***CE**			**3323.52**	**2614**	**95.07**	**26**	**.00**	**43.07**
4. Habitual sleep efficiency & 7. Daytime dysfunction	ACE	3832.22	2580	3860.95	2603	28.73	23	.19	-17.27
	ADE			3857.28	2603	25.06	23	.35	-20.94
	***AE**			**3860.95**	**2606**	**28.73**	**26**	**.32**	**-23.27**
	CE			3873.18	2606	40.96	26	.03	-11.04
5. Sleep disturbances & 7. Daytime dysfunction	ACE	3382.27	2601	3411.66	2624	29.39	23	.17	-16.61
	ADE			3408.68	2624	26.41	23	.28	-19.59
	***AE**			**3411.66**	**2627**	**29.39**	**26**	**.29**	**-22.61**
	CE			3426.10	2627	43.83	26	.02	-8.17

Note. * = best fitting model; -2LL = minus twice the log-likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in -2LL between the saturated model and the genetic model; Δ*df* = change in degrees of freedom between the saturated model and the genetic model; *p* = probability; AIC = Akaike's information criterion (calculated as $\Delta\chi^2 - 2\Delta df$); A = genetic influence; C = shared environmental influence; D = non-additive genetic influence; E = non-shared environmental influence. All analyses were obtained from Mx incorporating a weight to account for selection bias and attrition. Component 6 (use of sleeping medication) was excluded from all analyses due to the low frequencies in some categories of scores.

The parameter estimates from the best fitting bivariate genetic models were selected for interpretation and are presented in **Table 3.11**. The top half of the table indicates the genetic and environmental correlations on the associations between sleep components. For most associations, the bivariate genetic correlations (*rA*) were of moderate to high magnitude (9 of 15 correlations were ≥.50). For example the genetic correlation between 'subjective sleep quality and daytime dysfunction' was 54%$_{[95\%\ CI's,\ .33\text{-}.75]}$, suggesting substantial overlap in the genes influencing one component of sleep and those influencing another. For the 3 associations where shared environment was included

in the models, there was small to moderate overlap in these influences between components (*rC* = .18, .51 and .70). As compared to the *rA*, the non-shared environmental correlations (*rE*) were somewhat lower (11 of 15 correlations were ≤.40). For example, the non-shared environmental correlation between 'sleep latency and sleep duration' was $34\%_{[.23\text{-}.43]}$ suggesting that this source of influence was in general, more component-specific.

The bottom half of **Table 3.11** indicates the proportion of the phenotypic correlations accounted for by A, C, and E. The parameter estimates indicate that genetic influences accounted for between 37%-98% of all associations (with the exception of the 3 associations where CE models provided the best fit). In general, genetic influence accounted for roughly half of the associations (accounting for over 40% in 11 of 15 correlations) indicating that genes were partially responsible for the co-occurrence of any two phenotypes in all associations. For example, genes accounted for $59\%_{[.33\text{-}.83]}$ of the variance in the association between 'subjective sleep quality and habitual sleep efficiency'. For the three associations where genetic influence was excluded from the models, shared environment accounted for a small to moderate proportion of covariance (11%, 19% and 42%). Non-shared environmental influence explained a substantial proportion of covariance for the majority of the associations (ranging from 2%-89%; ≥40% in 13 of 15 correlations).

Table 3.11. Parameter estimates (with 95% confidence intervals) from the best fitting bivariate genetic models

	1. Subjective Sleep Quality	2. Sleep Latency	3. Sleep Duration	4. Habitual Sleep Efficiency	5. Sleep Disturbances	7. Daytime Dysfunction
1. Subjective Sleep Quality	/	*rA* .69 (.43 – .97) *rE* .54 (.41 – .65)	*rA* .68 (.41 – .96) *rE* .35 (.19 – .50)	*rA* .74 (.45 – 1.00) *rE* .29 (.12 – .46)	*rA* .57 (.33 –.80) *rE* .42 (.25 – .56)	*rA* .54 (.33 – .75) *rE* .35 (.21 – .50)
2. Sleep Latency	A .37 (.18 - .56) E .63 (.44 – .82)	/	*rC* .18 (-.27 – .55) *rE* .34 (.23 – .43)	*rA* .74 (.34 – 1.00) *rE* .35 (.20 – .49)	*rA* .82 (.54 – 1.00) *rE* .32 (.16 – .46)	*rA* .55 (.25 – .92) *rE* .23 (.07 – .38)
3. Sleep Duration	A .53 (.29 – .75) E .47 (.25 – .71)	C .11 (-.14 – .36) E .89 (.64 – 1.14)	/	*rC* .51 (.16 – .66) *rE* .62 (.53 – .71)	*rA* .62 (.32 – .99) *rE* .01 (-.12 – .19)	*rA* .27 (-.01 – .53) *rE* .26 (.09 – .43)
4. Habitual Sleep Efficiency	A .59 (.33 – .83) E .41 (.17 – .67)	A .43 (.16 – .68) E .57 (.32 – .84)	C .19 (.04– .25) E .81 (.75 – .95)	/	*rC* .70 (.35 – 1.00) *rE* .27 (.15 – .39)	*rA* .37 (.06 – .71) *rE* .12 (-.06 – .30)
5. Sleep Disturbances	A .48 (.25 – .70) E .52 (.30 – .75)	A .54 (.31 –.77) E .46 (.23 – .69)	A .98 (.51 – 1.58) E .02 (-.58 – .49)	C .42 (.20 – .66) E .58 (.34– .80)	/	*rA* .42 (.18 – .66) *rE* .42 (.25 – .58)
7. Daytime Dysfunction	A .55 (.31 – .78) E .45 (.22 – .68)	A .55 (.23 – .85) E .45 (.15 – .77)	A .41 (-.02 – .80) E .59 (.20 – 1.02)	A .64 (.12 – 1.19) E .36 (-.19 – .88)	A .42 (.17 – .66) E .57 (.34 – .83)	/

Note. Above diagonal: Bivariate Correlations *rA, rC, rE*; Below diagonal: Proportion of phenotypic correlation due to A, C and E. All analyses were obtained from Mx incorporating a weight to account for selection bias and attrition. Component 6 (use of sleeping medication) was excluded from all analyses due to the low frequencies in some categories of scores.

3.5 Discussion

The aim of this chapter was to address the first set of research questions posed in the introduction of this thesis. Specifically, this chapter examines (i) the extent to which genes and environments influence global sleep quality as measured by the PSQI as well as the individual components of sleep quality encompassed by this measure; (ii) the

phenotypic overlap between these components, with a focus on the clusters identified in previous research (Cole, et al., 2006); (iii) the extent to which genetic and environmental contributions overlap for different combinations of phenotypes; and (iv) the magnitude to which genetic and environmental influences contribute to the associations between components. The main findings here were that the contribution of genetic and environmental influences to the individual components of sleep quality varied somewhat between components, and most notably that genetic factors were not important for 'sleep duration'. Furthermore, the individual components of sleep quality were significantly associated, but the extent to which genes and environments explained these associations differed between clusters. Specific discussion of the phenotypic associations, and the genetic and environmental influences on global sleep quality, the individual components and the associations between them, is presented below followed by an outline of the limitations specific to this study.

3.5.1 Frequencies of sleep disturbances

The mean global sleep quality score in the present sample was 5.66 (SD = 3.01). Although scores above 5 typically indicate the presence of a clinically significant sleep disturbance (Buysse, et al., 1989), this score is in line with previous general population samples which have measured sleep quality using the PSQI. For example, in a population-based sample of 3403 adults with a mean age of 51 years in Japan, the mean PSQI score was 4.9 (Hayashino, et al., 2010); in a community study of 4173 adults aged between 18 and 65 years in Germany the mean PSQI score was 5.01 (Stein, Belik, Jacobi, & Sareen, 2008); and in a community sample of 401 adults aged between 18 and 68 years in the UK the mean PSQI score was 5.44 (Wood, Joseph, Lloyd, & Atkins, 2009).

Contrary to much of the previous literature (for example, see Ohayon, 2002, for a review) there was no evidence for sex differences in global sleep quality. Whilst this finding was unexpected, this result conforms with other reports which have not found evidence for statistically significant sex differences in global sleep quality score measured by the PSQI (Carpenter & Andrykowski, 1998; Driscoll, et al., 2008; Valentine, et al., 2009; Valladares, Eljammal, Motivala, Ehlers, & Irwin, 2008). However, it should be noted that, when assessing the individual components, there appeared to be a greater proportion of females reporting moderate/severe symptoms of 'sleep disturbances' than males. This finding is in line with numerous epidemiological studies which report a female bias in terms of the severity of sleep disturbance type symptoms (Zhang & Wing, 2006). In particular, Lichstein and colleagues reported a significantly greater number of awakenings (analogous to sleep disturbances measured here) in females as compared to males (Lichstein, et al., 2004) (although the authors note that, whilst significant, the reported sex differences were small). However, the authors also reported that females had significantly worse sleep efficiency than males, a finding which did not reach statistical significance in the present study. However, it should be noted that here the component severity categories were used rather than the raw scores from the individual scale items. Thus, utilising the component scores of the PSQI may not necessarily be the optimum method of assessing quantitative measures such as sleep efficiency.

3.5.2 Genetic and environmental influences on global sleep quality and the individual components of sleep quality

The magnitude of genetic and environmental influences on global sleep quality was consistent with previous reports (Heath, et al., 1990; Partinen, et al., 1983)

demonstrating that additive genetic influences accounted for a moderate amount of variance, with the remaining variance due to the nonshared environment. This finding adds to the small body of twin literature on the heritability of global sleep quality, and confirms that results using the PSQI to assess sleep quality are almost exactly the same as studies assessing sleep quality using a somewhat crude measure. In addition, there was no evidence for significant differences between males and females in the heritability of global sleep quality (or the individual components). This is contrary to a recent twin study in which sleep quality in females was found to be more heritable than in males (Paunio, et al., 2009). However, it should be noted that other studies assessing the heritability of sleep quality have not generally assessed differences in heritability between the sexes. As such, further studies investigating sex differences for sleep quality are essential in order to determine whether males and females do differ with regards to the magnitude of genetic and environmental influences on this phenotype.

For the individual components of sleep quality, genetic influences (both additive and non-additive effects) were important for the majority of components, although the magnitude of genetic influence varied somewhat between them. The estimates presented here (genetic influence ranging from 23%-50%) are in accordance with previous twin studies focusing on other individual aspects of sleep, such as daytime sleepiness, (Carmelli, et al., 2001), sleep quality (Heath, et al., 1990; Partinen, et al., 1983), sleep pattern (de Castro, 2002); and sleep latency and disturbance (Heath, et al., 1990). Although this study looks at sleep phenotypes in the normal range, these results suggest that some individuals may be genetically sensitive to developing problems with sleep. The finding reported here that genes did not influence sleep duration (assessed as a univariate trait) was unexpected as other studies report a strong genetic influence on 'sleep duration' (Heath, et al., 1990; Partinen, et al., 1983). A possible explanation for this discrepancy could be the age of the participants. Sleep patterns and difficulties are

affected by age (for example, Carrier, et al., 1997; Gregory & O'Connor, 2002; Jones, et al., 2007; Kramer, Kerkhof, & Hofman, 1999), and so it is possible that sleep duration may be more variable in young adults than at other ages. For example, Partinen and colleagues (1983) dichotomised their sample into those aged 18-24 years and those 25 years and over. The authors found that genetic influence on 'sleep duration' appeared to be smaller in those aged 18-24 years compared to those aged 25+ years. As such, it is possible that for younger participants, such as those in the present study, genes play a less prominent role for this phenotype. In support of this, Gedda and Brenci (1979) found that genetic influences were not important for sleep duration in children aged between 6-8 years, but of some importance in teenagers (16-18 years). Likewise, Gregory and colleagues (2006) found no evidence for genetic influence on child reported sleep duration in a small sample of school-aged children. It is thought that this occurs due to the greater importance of family-wide environmental experiences present in younger individuals. As such, it appears that estimates of genetic influence on sleep duration vary as a function of the developmental time period encapsulated by the sample. This may also be true in young adulthood. Furthermore, many of our participants were studying at university (40%). At university there is potentially social pressure to stay out and go to bed late, and the possibility of a less rigid routine compared to individuals in full-time work. Thus, a tentative suggestion is that such environmental pressures may have attenuated the impact of genes on sleep length. Indeed, in the present study, the students – as compared to the non-students went to bed significantly later (mean time = 11:56pm, SD = 1 hour 19 minutes vs. mean time = 11:15pm, SD = 1 hour 21 minutes, respectively; $t(1530) = 9.68$, $p < .001$) and slept significantly longer (mean time = 7 hours 38 minutes, SD = 8.4 minutes vs. mean time = 7 hours 25 minutes, SD = 16.2 minutes, respectively, $t(1523) = -3.32$, $p < .001$), suggesting that perhaps the non-shared environment was a significant source of

influence for these participants. A further possible explanation for our lack of genetic influence on sleep duration is that, of all the components of the PSQI, sleep duration is likely to be under the most voluntary control. It can be difficult to control sleep latency, efficiency, quality, disturbances, etc., but often one can control the amount of sleep one gets. Thus, the smaller influence of genes, especially with regards to this age group, may reflect the fact that one can voluntarily give in to the social pressures that may be much stronger here than in other age groups, and thus the impact of genes may be attenuated.

A point worthy of consideration regards the type of genetic effects observed for the individual components of sleep. Additive genetic effects were important for sleep latency, habitual sleep efficiency and sleep disturbances, yet non-additive genetic effects were important for the subjective sleep quality component and daytime dysfunction. These findings provide an insight into the possible genetic mechanisms which may be at play. Future research into these mechanisms will reinforce the findings presented here. However, it should be highlighted that as these findings are preliminary it may be best to interpret the results in terms of 'broad sense heritability', combining both additive and non-additive effects, as it is not possible to make further speculations as to the exact mechanisms at play within this dataset.

Shared environmental influences were non-existent for global as well as the individual components of sleep quality except for sleep duration. This is in line with a previous twin study of sleep which has demonstrated that the familial environment becomes less influential to sleep-wake behaviour when twins live independently from one another (de Castro, 2002). This is also consistent with a substantial amount of other behavioural genetic research focusing on other phenotypes, where the contribution of the shared environment has been found to often be indistinguishable from zero (Burt, 2009; Plomin & Daniels, 1987). This is generally thought to occur as individuals have a

tendency to seek out novel environments in adulthood, and so the effect of the non-shared environment is much more substantial. However, shared environmental influence was important for 'sleep duration', accounting for 26% of the variance in the phenotype. This suggests that, even in young adults, family-wide influences are important for determining how long one sleeps. For twins living in the same home, it could be suggested that shared influences such as living near a noisy road, or having strict rules about going to bed and getting up at a set time contribute to this.

Non-shared environmental influences were important for global as well as the individual components of sleep quality assessed here, and explained the majority of variance in all cases. Although non-shared environmental influence includes error, this finding may suggest that identifying individual-specific environmental influences that affect sleep quality may lead to a better understanding of normal sleep habits and, potentially, problems. Possible non-shared environmental factors could include early childhood adversities and family conflict, which have been found to predict later poor sleep quality and insomnia in adulthood (Gregory, Caspi, Moffitt, & Poulton, 2006; Koskenvuo, Hublin, Partinen, Paunio, & Koskenvuo, 2010); relationship issues (Ohayon, 1996); negative life events (Bernert, Merrill, Braithwaite, Van Orden, & Joiner, 2007); or ill health and physical pain which are often associated with poor sleep (Ohayon, Caulet, & Guilleminault, 1997).

3.5.3 Phenotypic associations between components of sleep quality

Associations were found between all components of sleep quality, of varying magnitude, suggesting that the components to some extent measure an underlying construct of 'sleep quality' - supporting the use of the global score of the PSQI. Consistent with Cole and colleague's (2006) findings, the strongest associations were between, 1) 'subjective sleep quality and sleep latency'; and 2) 'habitual sleep

efficiency and sleep duration', though there was only a moderate association between 'sleep disturbances and daytime dysfunction' (which were also moderately associated with 'subjective sleep quality'). These findings support Cole and colleague's proposed structure of the PSQI, and suggest that overall sleep quality relies on a short sleep onset latency, few disturbances, and little daytime dysfunction. However, the far from perfect concordance between components suggests some degree of specificity between them. The weakest association was found between 'habitual sleep efficiency and daytime dysfunction'. This suggests that less efficient sleep does not necessarily impede daytime functioning.

3.5.4 Genetic and environmental influences on the associations between components of sleep quality

The present study aimed to examine *why* different aspects of sleep quality co-occur. For most associations, genes were important determinants of the associations between components. For example, a large proportion of the association between 'habitual sleep efficiency and daytime dysfunction' was accounted for by genes (despite the phenotypic correlation being small, $r = .22$), and there was substantial overlap in the genes influencing the majority of the components. As such, similar genes influenced the co-occurrence of these phenotypes. Finding genetic overlap between problems suggests that once genes have been found that influence one aspect of sleep, those genes may be worthwhile exploring in relation to other aspects of sleep with which it is associated. Based on this, genes may be preferentially sought for those factors that show the highest genetic overlap. Although specific genes were not identified in this study, possible candidate genes could be proposed from previous research (for example, see Brummett, Krystal, Ashley-Koch, et al., 2007; Serretti, et al., 2003).

For Cole's three factors, the influence of genes and degree of overlap was variable, suggesting that the grouping of components within these factors occur for different reasons. As an example, for the 'sleep efficiency' factor (encompassing 'sleep duration' and 'habitual sleep efficiency'), genes accounted for 0% of the phenotypic correlation between the phenotypes comprising this factor, whereas for the 'perceived sleep quality' factor (encompassing 'subjective sleep quality' and 'sleep latency'), there was substantial overlap in the genetic influences attributable to both phenotypes in this association, and overall genes accounted for 37% of the phenotypic correlation. Accordingly, the results presented here to some extent support the factor structuring of the PSQI proposed by Cole et al., although some associations show genetic specificity.

One finding worth further consideration is the association between 'sleep disturbances and sleep duration'. The univariate analysis indicated that genetic influence was absent for 'sleep duration', however genetic factors explained almost all of the association with sleep disturbances. Incorporating variables into a bivariate model can alter the univariate estimates on individual traits as estimates consider both phenotypes simultaneously. Indeed, the univariate genetic influence on 'sleep duration' was estimated at .34 in the bivariate model (and the genetic correlation was .62), which explains why there was considerable genetic influence on the association with this and the other phenotypes. Too much weight should not be placed on this finding as the phenotypic correlation between 'sleep disturbances and sleep duration' was small.

Overall, the finding that the associations between phenotypes were moderate, and that the overlap in the genetic influences accounting for most phenotypic associations was substantial, suggests that the components of the PSQI may be influenced by shared genes. Large genetic associations between different behaviours has been reported previously with regards to internalizing problems such as anxiety and depression (Middeldorp, Cath, Van Dyck, & Boomsma, 2005), and it is suggested that

'general genes' are important for both types of psychopathology (Eley, 1997). Thus, it appears that the 'generalist genes' hypothesis may be applicable to the components of sleep quality assessed here. As such, the data here support the use of the global score of the PSQI in studies assessing normative sleep patterns in healthy populations, since it appears that the components, stemming from similar genetic factors, may overall measure one underlying trait. However, it must also be noted that the shared genetic correlations between components, in all cases, were less than unity, suggesting that there are, to some extent, unique genetic effects at play between components.

Environmental factors were also important in explaining the co-occurrence of phenotypes. For most associations, shared environmental influences were negligible, the main influence being the non-shared environment. Non-shared environmental influences, although important for all associations, were of greatest magnitude for the association between 'sleep latency and sleep duration'. It is possible that difficulty falling asleep and disruptions from sleep could be influenced by environmental factors, such as noise, temperature and disruption from others. There was partial overlap, of varying magnitude, in the non-shared environmental influences affecting all combinations of phenotypes, suggesting that the environmental influences on the individual components of the PSQI were more variable and component specific than were genetic influences - which showed much greater similarity across components. This, again, follows the pattern often seen in other areas of developmental psychopathology, where general genes, but specific environmental influences account for the overlap between phenotypes (Eley, 1997). This pattern was reflected in Cole's three factors also, where the influence of the environment, although significant in explaining the associations between phenotypes, was much more variable across factors. As such, the search for unique environmental influences on the individual aspects of sleep may be beneficial in identifying those at risk for poor sleep quality.

3.5.5 Possible implications for nosology

The findings presented in this chapter may be informative for the area of sleep research focussed on the nosology of insomnia and insomnia subtypes. There is much debate within the literature as to how to conceptualise insomnia and its possible subtypes (Edinger, et al., 1996; Edinger & Krystal, 2003). Some researchers conceptualise insomnia subtypes based on the diagnostic criteria for primary insomnia, including sleep onset insomnia, sleep maintaining insomnia, and insomnia with early morning awakening (Hohagen, et al., 1994). Recent research found moderate associations between subtypes categorised in this way, and that the subtypes were differentially associated with depression, suggesting that the subtypes were somewhat distinct (Yokoyama, et al., 2010). The present results, however, found a high correlation between sleep latency and sleep disturbances (which may be considered analogous to measuring sleep onset insomnia and sleep maintaining insomnia). There was also substantial genetic overlap between these components. Accordingly, the present results, genetically speaking, do not support the differentiation of sleep onset insomnia from sleep maintaining insomnia, suggesting that they may stem from a similar genetic background. In partial accordance with this, Ohayon and Bader (2010), investigated the determinant factors of the three main DSM-IV defined insomnia symptoms, difficulty initiating sleep, difficulty maintaining sleep, and non-restorative sleep. The authors found that difficulty initiating sleep and difficulty maintaining sleep appeared to share common predictive factors, whilst non-restorative sleep appeared to have a distinct profile. The authors suggest the possibility that the specific symptom, non-restorative sleep, may have a unique aetiological profile compared to that of other insomnia symptoms. Further research specifically focussed on addressing the aetiological overlap between insomnia symptoms and subtypes defined in this way will be useful in terms of further validating these distinctions. More recent and comprehensive classification

systems, such as the ICSD-2, divide insomnia into highly specific subtypes, including psychophysiological insomnia, idiopathic insomnia, inadequate sleep hygiene, and sleep-state misperception, to name a few (American Academy of Sleep Medicine, 2005). The DSM-IV, however, simply differentiates between primary insomnia, insomnia related to another mental disorder or organic factor, and substance induced sleep disorder (American Psychiatric Association, 1994). Research aimed at investigating the underlying causes of the primary symptoms of each of these subtypes will aid in determining the best method for subtyping insomnia. Such research may be useful for refining diagnostic criteria for the forthcoming edition of the DSM.

In addition to potentially informing the subtyping of insomnia, the present results may provide information for subtyping sleep quality. The lowest genetic correlations, and hence, the highest degree of aetiological specificity, was between the components sleep latency and sleep duration; habitual sleep efficiency and sleep disturbances, (where bivariate models excluding genetic effects provided the best fit in both cases); and sleep duration and daytime dysfunction. The three highest genetic correlations were between 'subjective sleep quality and habitual sleep efficiency'; 'sleep latency and habitual sleep efficiency'; and 'sleep latency and sleep disturbances'. Accordingly, these results perhaps suggest that sleep quality could be subdivided into categories distinguishing between components based on degree of 'sleep fragmentation' type symptoms (including sleep latency and sleep disturbances), overall sleep quality (including subjective sleep quality and habitual sleep efficiency), and considering sleep duration and daytime dysfunction as separate entities.

3.5.6 Limitations

There are a number of limitations relevant to all analyses outlined in this thesis that should be considered when interpreting all findings presented. These more general

limitations are discussed in detail in Chapter 8 (section 8.3). Here limitations specific to the analyses of the present chapter are discussed. The first limitation specific to this study regards statistical analyses. Using ordinal data can reduce the power to detect genetic and environmental components of variance. Furthermore, the computationally intensive integration method used limits the number of variables that can be simultaneously analysed. This means that it was unfeasible to combine all component sleep scores into one multivariate model, which is favoured over conducting multiple bivariate models as it increases power. Additionally, in several of the bivariate associations, confidence intervals on the parameters were wide and spanned zero. This largely reflects the sample size and consequent power limitations. As such, these findings should be considered as preliminary and interpreted accordingly. This highlights the need for replications in much larger samples.

A second limitation regards the age of the sample. Because the age range of our sample was limited to 18-27 years, with 90% of the sample clustering around 18-22 years of age, we were unable to investigate age differences in relation to the heritability of the components of sleep quality. Such an investigation may be valuable since it is known that sleep changes with age (Gregory & O'Connor, 2002; Kramer, et al., 1999). Further research from this study will be necessary in order to determine whether the relative contribution of genes and environments on these components and their overlap fluctuates over time. However, the limited age range studied here reduces genetic heterogeneity and so provides an accurate description of the relative impact of genes and environments on sleep in the age group under study.

A third limitation concerns the use of sleeping medications within our sample. Although the component ‘use of sleep medication’ was not analysed in its entirety, all individuals (including those who claimed to use sleeping medications frequently) were included in the analyses of the other components. Although only a small percentage of

the sample were using sleeping medications frequently (fewer than 3%), it is possible that their inclusion could artificially inflate the non-shared environmental estimates, since one twin's sleep may have differed to their co-twin as a result of one twin using medications. However, there was no support for this hypothesis when the analyses were re-run after excluding all of those who took sleep medicines (unreported), the results of which were substantially similar to those reported.

3.5.7 Conclusion

In conclusion, the results from this chapter suggest that there is substantial overlap in the genetic influences on the individual components of sleep quality encompassed by the PSQI. This points to the conclusion that these components to some extent measure an underlying construct of sleep quality, supporting the use of the global score. In addition, non-shared environmental influences accounted for the majority of variance in both global sleep quality as well as the individual components. Although standard twin analyses such as these provide us with no information regarding the contribution of specific non-shared environmental influences, alternative twin designs have the ability to inform us about the specificity of the non-shared environment. It is to this issue that we now turn in Chapter 4.

3.6 Acknowledgments

A manuscript based on the analyses of this chapter has been published and I thank my co-authors for their contribution.

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Chapter 4: Non-Shared Environmental Influences on Sleep Quality: A Monozygotic-Twin Differences Approach

4.1 Overview

Research has consistently demonstrated that environmental influences are important for explaining the variability in sleep quality observed in the general population. Although there is substantial evidence assessing associations between sleep quality and a host of environmental variables, it is possible that their effects are mediated by genetic influence. This chapter uses the MZ twin differences design to examine whether the associations between a host of candidate 'environmental' measures and global sleep quality are mediated via genetic influences (or indeed the shared environment), or whether there is a purely non-shared environmental effect at play. When controlling for genetic and shared environmental effects, within monozygotic twin-pair differences in sleep quality were associated with within monozygotic twin-pair differences in general health for males (β = 1.56, p <.001) and relationship satisfaction for females (β = 1.01, p <.05). These results suggest that the associations between these variables have a significant non-shared environmental effect. However, for the remaining environmental measures assessed, the results suggest that these seemingly 'environmental' influences are actually in part dependent on genetics and/or the shared environment. These findings give insight into how specific environments affect sleep and the possible mechanisms behind these associations.

4.2 Introduction

There is now a growing body of evidence for genetic influence on sleep related phenotypes (for example, see Gregory & Franken, 2009), a finding which is supported by numerous twin studies (e.g. Heath, et al., 1990; Partinen, et al., 1983) including the results of Chapter 3, demonstrating that separate components of sleep quality are in part influenced by genetic factors. What is particularly interesting about twin studies, however, is that they can tell us as much about the environment as they can about genetics. Indeed, the aforementioned twin studies highlighted that, in addition to genetics, experiences specific to each twin (non-shared environmental influences) appear to influence and account for a substantial proportion of variance in sleep quality, assessed as a global construct as well as when the individual components are examined separately; whereas shared environmental influences by contrast appear to contribute little (with the exception of sleep duration). Explicit investigation of these distinct types of environmental influence (shared vs. non-shared) is key to understanding the mechanisms through which the environment affects behaviour.

However, standard quantitative genetic analysis, as in Chapter 3, tells us nothing about the specific environmental influences at play. One method for examining specific non-shared environmental influences on traits is to use only monozygotic (MZ) twins. Because MZ twins share 100% of their genetic make-up as well as their shared environment, any differences between them must be due to the non-shared environment (and measurement error). In standard twin analyses, identifying non-shared environmental factors that function independently of genetics and the shared environment is complicated by the fact that genetic factors may influence, or interact with, the environment (for an overview of gene-environment interplay see Chapter 1, section 1.6.4). This hypothesis is confirmed by studies demonstrating that genetic factors often influence exposure to certain environmental conditions – a concept termed

gene-environment correlation (Plomin, et al., 2008). Thus, many measures traditionally thought of as 'environmental' are actually in part genetically driven. Indeed, Kendler and Baker (2007) demonstrated that a host of environmental measures, including stressful life events, parenting, social support, peer interactions and marital quality are around 30% heritable. This means that we may select our environments based on our genetic predispositions. Furthermore, genes and environments may interact to bring about behaviour. Gene-environment interaction may mean that an individual is genetically sensitive to a particular trait or disorder, but that symptoms are evident only in the presence of an identified environmental stressor (for further discussion, see Chapters 1 and 5). Thus, gene-environment correlation and interaction make it difficult to identify purely non-shared environmental components that are independent of genetics or the shared environment. The MZ twin differences design, however, assesses the degree of dissimilarity between MZ twins on a measured environmental variable and an outcome variable which allows us to disentangle the interplay between genes and environments to determine purely non-shared environmental components contributing to the outcome. The MZ twin differences design has been used to determine specific non-shared environmental effects for a number of traits, for example behavioural development and adolescent depression (Asbury, Dunn, Pike, & Plomin, 2003; Caspi, et al., 2004; Liang & Eley, 2005), and is a simple yet powerful method of determining twin discrepancy that is independent of genetics and the shared environment (Pike, Reiss, Hetherington, & Plomin, 1996).

Of course in order to determine whether the environment has a purely non-shared environmental contribution to a phenotype of interest, candidate environmental influences must first be selected for investigation. Prior research has implicated a number of 'environmental' influences on sleep quality that may be worthy candidates for analysis. For example, several studies have highlighted the negative effects of

stressful life events on sleep quality (Lavie, 2001; Mezick, et al., 2009; Sadeh, 1996; Vahtera, et al., 2007). Within the life events literature, a common conceptualisation of life events is to categorise them as dependent or independent according to the perceived controllability of such events (Brown & Harris, 1978). Dependent negative life events can be defined as those that an individual has some degree of control in bringing about (examples included in this category are financial or relationship problems). Independent negative life events are defined as those not influenced by an individual's behaviour (examples considered in this category include death of a relative or having something valuable lost or stolen). Indeed, a variety of negative life events have been associated with intra-individual variability in sleep duration and fragmentation (Mezick, et al., 2009), sleep disturbances (Lavie, 2001), and sleep onset disturbances (Vahtera et al., 2007). Another study shows a dose-response relationship such that individuals experiencing more family conflict experience poorer sleep (Gregory, Caspi, et al., 2006). Although it is clear that both controllable and uncontrollable events have negative consequences on sleep it is possible that these distinct types of negative life event are associated with sleep differentially. For example, the feelings of responsibility involved in creating dependent negative life events could hinder sleep through the worry and cognitive rumination of the negative events to a greater extent than do independent negative life events. Indeed heightened cognitive arousal is known to disrupt sleep (Harvey, 2002). Other phenotypes, such as depression, have been found to be associated differentially with dependent and independent negative life events (Cui & Vaillant, 1997; Liang & Eley, 2005).

Other candidates for study include demographic differences and relationships. Epidemiological data suggest that sleep difficulties are more prevalent among individuals with a low income, low educational attainment, and those unemployed (Ford & Kamerow, 1989; Grandner, et al., 2010; Ohayon, 1996, 2009a). They are also more

prevalent in separated/divorced and widowed individuals as compared to married couples (Ford & Kamerow, 1989; Grandner, et al., 2010; Hale, 2005). Furthermore, marital satisfaction has been found to be associated with fewer sleep disturbances in women (Troxel, Buysse, & Hall, 2009) and so it appears that the quality of romantic relationships, in addition to relationship status, may be an important determinant of sleep. Given the apparent importance of relationships on sleep it may also be important to assess the quality of peer relationships and friendships. In children bullying interferes with sleep (Williams, Chambers, Logan, & Robinson, 1996), and low connectedness with peer groups is associated with subjective insomnia in adolescents (Yen, Ko, Yen, & Cheng, 2008). Similarly, in adults workplace bullying is associated with sleep disturbances (Lallukka, Rahkonen, & Lahelma, 2010; Neidhammer, David, Degioanni, Drummond, & Philip, 2009). Accordingly, investigation of friendships appears warranted. Additionally, the depression literature shows that affiliation with deviant peers is associated with increased depressive symptoms (Fergusson, Wanner, Vitaro, Horwood, & Swain-Campbell, 2003). This relationship is in part mediated by the increase in deviant behaviours that occurs in individuals affiliating with deviant peers. Research from our team has demonstrated that sleep problems are associated not only with depression (Gregory, Buysse, et al., 2011), but also with deviant behaviours (Barclay, Eley, Maughan, Rowe, & Gregory, 2010). Because of these associations it is worth considering whether peer groups and affiliations with deviant peers also influence sleep quality. A final candidate for study is general health. Poor sleep is associated with poor general health (for example, see Briones, et al., 1996; Finn, Young, Palta, & Fryback, 1998; Gangswisch, et al., 2010), and so it is possible that differences in health are associated with differences in sleep between twins. In addition, consideration of possible sex effects is also worthy of investigation. Although the results of Chapter 3 found no evidence for significant sex differences in global sleep quality, sleep is

typically known to differ between males and females (Ohayon, 2002). As such it is possible that not only does sleep itself differ between the sexes, but that the influences that affect sleep are also sex dependent (in the standard twin design it is not possible to investigate qualitative sex differences in the non-shared environment as these influences are unique to each individual and so it does not make theoretical sense to equate them).

Although the extant literature demonstrates that numerous traditionally viewed 'environmental' factors are associated with sleep, as previously discussed many have been shown to be under genetic influence (Kendler & Baker, 2007). Thus, whether these associations are also influenced by a purely non-shared environmental component (in addition to the proportion explained by genetics) is in question. Investigating this issue will shed light on the mechanisms by which these factors are associated with poor sleep. Accordingly, in this study the MZ twin differences design was used to address the second set of research questions of this thesis. Specifically, it investigates (i) whether there are significant associations between sleep quality and a range of candidate non-shared environmental factors (including negative life events, [dependent and independent], demographic characteristics, aspects of relationships [both romantic and with peers] and general health); and (ii) whether there is a purely non-shared environmental component to these associations (i.e. do these associations remain significant even after controlling for genetic and shared environmental factors). Furthermore, differences between males and females were tested in order to determine whether the effects were sex-specific. Sleep quality in the present study (and indeed throughout the remainder of this thesis) was assessed using the global score. Examination of the global score is perhaps the most accurate conceptualisation of 'sleep quality', given that this is the PSQIs most common usage, and because the significant phenotypic correlations between components in Chapter 3 suggested that these components were to some extent measuring one underlying construct.

4.3 Method

4.3.1 Participants

Data from all complete monozygotic (MZ) twin pairs (n=190 complete pairs) from Wave 4 of the G1219Twins study were used in analyses (Mean age=19.8 years [SD=1.26], range=18-22 years; 65.8% female).

4.3.2 Measures

4.3.2.1 Sleep quality

Sleep quality was assessed by the global score of the Pittsburgh Sleep Quality Index – described in Chapter 3 (section 3.3.2). In the sample used in this study, the PSQI global score yielded satisfactory internal reliability (Cronbach's alpha (*a*) = .70).

4.3.2.2 Dependent and independent negative life events

Negative life events were assessed using items from the 'List of Threatening Experiences' (Brugha, Bebbington, Tennant, & Hurry, 1985), and the 'Coddington Stressful Life Events Scale' (Coddington, 1984). Participants were required to respond to these checklists by indicating whether or not they had experienced a particular negative life event in the last year. Twenty one items were summed to give a score of total life events, which were further subdivided into 13 items assessing dependent negative life events (e.g. break up of a steady relationship) and 8 items assessing independent negative life events (e.g. death of a parent). Dependent and independent negative life events were classified according to whether it is likely that their occurrence is the consequence of an individual's behaviour as suggested by Brown and Harris (1978). This distinction between life events has been used in previous studies (Silberg,

et al., 2001) as well as another paper from the G1219 study (Liang & Eley, 2005). The items included in these scales are outlined in **Table 4.1**.

Table 4.1. Items included in the dependent and independent negative life events scales taken from the 'List of Threatening Experiences' and the 'Coddington Stressful Life Events Scale'

Dependent Negative Life Event Scale
1. Separation due to marital difficulties
2. Serious problem with a close friend, neighbour or relative
3. Problems with police or court appearance
4. Unemployed or seeking work for more than one month
5. Suspension/expulsion from college or university
6. Have become involved in drugs
7. Had a major financial crisis
8. Break up of a steady relationship
9. Failed end of year exams
10. Start of a new problem between you and your parents
11. Been sacked from a job
12. Been invited by a friend to break the law
13. Have failed to achieve something you really want
Independent Negative Life Events Scale
1. Been in hospital with a serious illness or injury
2. A parent hospitalized for a serious illness or injury
3. Death of a second degree relative (e.g. grandparent)
4. A sibling hospitalized for a serious illness or injury
5. Death of a parent
6. Had something valuable lost or stolen
7. Death of a sibling
8. Death of a close friend

4.3.2.3 Education

Educational achievement was assessed by one question asking participants their highest UK qualification (ranging from none, GCSE, GNVQ, AS level, A level, BTEC, HND, degree). Higher scores indicated a higher level of education attained.

4.3.2.4 Employment

Participants were initially asked to indicate their employment status according to one of the following categories: unemployed, full-time student, employed full-time, part-time student/work, on gap year, on government benefit, or full-time parent. However, a large proportion of the sample (93.9%) fell into either the 'employed full-time' (48.4%) or 'full-time student' (45.5%) category, with only a few participants unemployed (n=13) or full-time parents (n=7). No participants fell into the remaining categories. As such employment status was re-categorised as 'employed full-time' vs. 'full-time student' as the number of cases in the other categories were considered too small and so were coded as missing for this analysis.

4.3.2.5 Relationship status

Participants were initially asked to indicate their relationship status according to one of the following categories: married, living with partner, engaged, living with partner and engaged, going steady, casual or single. Although typically previous research has assessed relationship status with a wider scope (e.g. divorced, widowed, etc. Ohayon, 2002) the participants in the present study were relatively young (aged between 18 and 22 years) and so only one was married and none were divorced/widowed. As such it was not possible to distinguish between these categories. As a large proportion of the sample fell into either the 'going steady' (33.2%) or '

single' (48.2%) category, relationship status was finally categorised as those who were 'single' vs. 'those in a romantic relationship' (49.7%) at the time of assessment.

4.3.2.6 Relationship satisfaction and cohesion

Relationship satisfaction and cohesion were assessed by items included in the subscales of the Dyadic Adjustment Scale (Spanier, 1976). The satisfaction subscale comprised 6 items tapping into aspects of the relationship such as happiness, and the cohesion subscale by 5 items assessing the extent to which the participant and their partner engage in activities together. The items included in these scales are outlined in **Table 4.2**. Participants were asked to respond to statements on 5 or 6 point scales, as outlined in **Table 4.2**. Generally, lower scores indicate better satisfaction and cohesion. These scales are widely used and have been found to have satisfactory validity and reliability (Spanier, 1976; Spanier & Thompson, 1982). In the present sample Cronbach's a = .77 and .67, for relationship satisfaction and cohesion, respectively.

Table 4.2. Items included in the relationship satisfaction and cohesion subscales of the 'Dyadic Adjustment Scale'

Relationship Satisfaction
1. How happy are you in your relationship?
How often would you say that the following occurs...?
2. You think that, in general, things between you and your partner are going well?
3. You and your partner get on each other's nerves?
4. You are your partner have an argument?
5. You regret you started the relationship?
6. You and your partner discuss or consider divorce, separation, or ending your relationship?

Table 4.2 (continued). Items included in the relationship satisfaction and cohesion subscales of the 'Dyadic Adjustment Scale'

Relationship Cohesion
How often would you say the following events occur between you and your partner...?
1. Have a stimulating exchange of ideas
2. Laugh together
3. Calmly discuss something
4. Work together on a project
5. Do you and your partner engage in any outside interests together?

Note. Relationship Satisfaction: Question 1 is coded as 0 = extremely unhappy; 1 = fairly unhappy; 2 = a little unhappy; 3 = happy; 4 = very happy; 5 = extremely happy; 6 = perfect. Questions 2-3 are coded as 1 = all the time; 2 = most of the time; 3 = more often than not; 4 = occasionally; 5 = rarely/never. Relationship Cohesion: Questions 1-4 are coded as 1 = daily; 2 = weekly; 3 = monthly; 4 = rarely; 5 = never. Question 5 is coded as 0 = none of them; 1 = very few of them; 2 = some of them; 3 = most of them; 4 = all of them).

4.3.2.7 Closest friendship quality

The quality of closest same-sex friendship was assessed by 21 items from the Network of Social Relationships Inventory (Furman, 1986). This measure comprised 7 3-item subscales tapping into a range of qualities of best friendships (e.g. affection, companionship, instrumental aid, intimacy/disclosure, nurturance, reliable alliance and support). Participants were asked to respond to questions such as, "How much do you talk about everything with this person?" with responses ranging from 'little/none', 'somewhat', 'very much', 'extremely', and 'the most'. Responses to all questions were combined to generate an overall mean measure of closest same-sex friendship quality. Higher scores indicate better quality of friendship. The individual scales have demonstrated good psychometric properties (Furman, 1986), and the global score in the

present sample demonstrated excellent internal reliability (a = .93). The items included in this scale are outlined in **Table 4.3**.

Table 4.3. Items included in the closest same-sex friendship scale from the 'Network of Social Relationships Inventory'

1. How much of your free time do you spend with this person?
2. How much does this person teach you how to do things that you don't know?
3. How much do you talk about everything with this person?
4. How much do you help this person with things she/he can't do by her/himself?
5. How much does this person like or love you?
6. How sure are you that this relationship will last no matter what?
7. How often do you turn to this person for support with personal problems?
8. How much do you play around and have fun with this person?
9. How much does this person help you figure out or fix things?
10. How much do you share your secrets and private feelings with this person?
11. How much do you protect and look out for this person?
12. How much does this person really care about you?
13. How sure are you that your relationship will last in spite of fights?
14. How often do you depend on this person for help, advice, or sympathy?
15. How often do you go places and do enjoyable things with this person?
16. How often does this person help you when you need to get something done?
17. How much do you talk to this person about things that you don't want others to know?
18. How much do you take care of this person?
19. How much does this person have a strong feeling of affection (loving /liking) toward you?
20. How sure are you that your relationship will continue in the years to come?
21. When you are feeling down or upset, how often do you depend on this person to cheer things up?

Note. Items are coded as 1 = little/none; 2 = somewhat; 3 = very much; 4 = extremely; 5 = the most.

4.3.2.8 Friendship quality

Friendship quality was measured by 5 items included in the Edinburgh Study of Youth Transitions and Crime (ESYTC) (Smith & McVie, 2003) and assessed "How often do your friends, (i) support you when you need them; (ii) fall out with you; (iii) put you down in front of others; (iv) make you feel confident; and (v) put pressure on you to do things you don't want to." Responses ranged from 1 = most days; 2 = at least once a week; 3 = less than once a week; and 4 = hardly ever/never. Scores from these items were combined (and where necessary responses were reversed) to provide a total score on friendship quality. Higher overall scores indicated poorer friendships. In the present sample a = .44.

4.3.2.9 Deviant peers/ Affiliation with deviant peers

The presence of deviant peers was assessed by asking participants whether their friends engaged in a number of deviant behaviours, including alcohol, tobacco or cannabis use, and whether they truanted or broke the law, as outlined by Fergusson and colleagues (2003). Responses were coded as 0 = none; 1 = one or two; 2 = some; 3 = most/all. Deviant peer affiliation was assessed by 3 items asking "How likely is it that you would still stay with your friends if they were getting you in trouble (i) with your family; (ii) at work/college; (iii) with the police?" as outlined in the ESYTC (Smith & McVie, 2003). Responses were coded as 1 = very likely; 2 = fairly likely; 3 = not very likely; 4 = not at all likely. Higher scores on both measures indicate greater problems.

4.3.2.10 General health

General health was assessed by one item assessing subjective general health using the question, "In general, how good would you say your health is now?" with responses on a 5-point scale ranging from 1 = Excellent to 5 = Poor, as outlined by Ware and Sherbourne (1992). General health has reliably been measured in this way in numerous studies, for example see (Troxel, et al., 2009).

4.3.3 Statistical analyses

4.3.3.1 Twin difference measure

In each pair of MZ twins, one twin was randomly assigned as 'Twin 1' and the co-twin as 'Twin 2'. A relative difference score was then calculated for sleep quality as well as each of the environmental measures by subtracting Twin 1's score on each measure from that of Twin 2's score on each corresponding measure. Because monozygotic twins share 100% of their genetic material as well as 100% of their shared environment, any differences between them must be accounted for by the non-shared environment (including measurement error). Thus, the MZ twin difference measure provides an unbiased estimate of twin discrepancy due to the non-shared environment (including measurement error) (Rovine, 1994).

4.3.3.2 Analyses in the monozygotic twin differences design

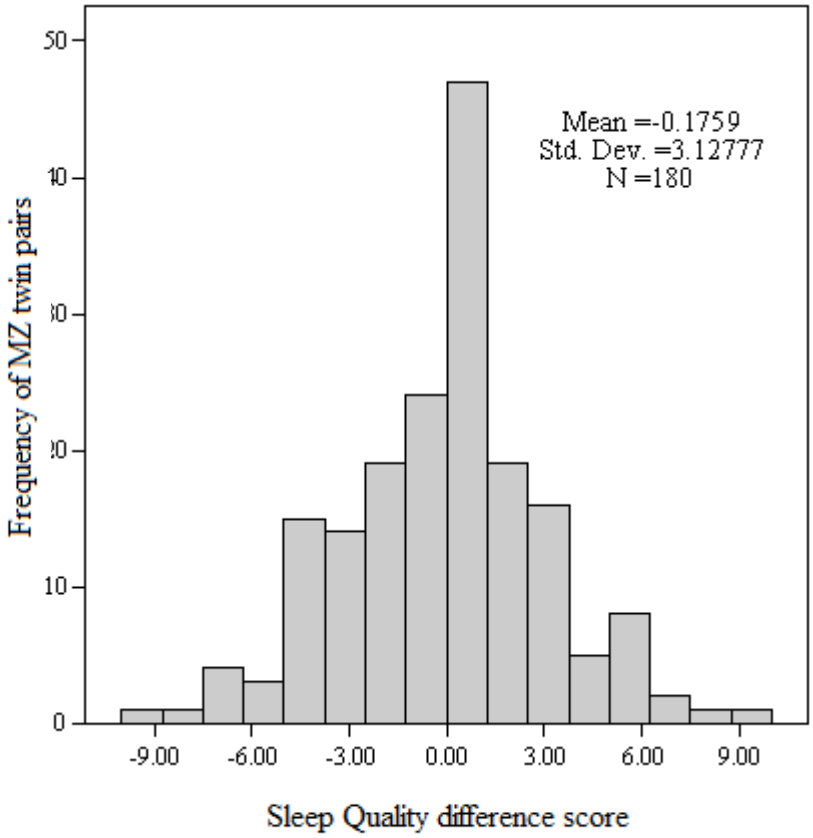
First, descriptive statistics and correlations between the environmental measures and sleep quality were calculated in SPSS. Second, a series of univariate linear regression analyses were run in STATA (Stata, 2002), to assess the contribution of each of the absolute environmental measures on absolute sleep quality scores. Third, a series

of univariate linear regression analyses were run using MZ twin difference scores for each of the environmental measures to predict MZ differences in sleep quality. The MZ differences analysis, using a measure of twin discrepancy, thus controls for the effects of genetics and the shared environment. All regression analyses were run separately for males and females in order to determine whether the effects were sex specific. The regression analyses also considered effects of age as well as clustering within the sample due to the inclusion of MZ twins, and thus non-independence of observations, by using the Robust(cluster) command in STATA.

4.4 Results

Descriptive statistics including MZ twin pair correlations are presented in **Table 4.4**. There were no significant sex differences in sleep quality ($t(366) = 1.15$, $p=.25$). The distribution of the MZ twin difference scores for sleep quality is shown in **Figure 4.1**. The mean of the MZ difference scores generally approximate zero since the random assignment of twins ensures that cases where twin 1 scores higher than twin 2 are cancelled out by cases in which twin 2 scores higher than twin 1. What is important is the distribution of these scores. Clustering around zero demonstrates similarity between twins whereas deviations from zero demonstrate the presence of differential sleep quality due to the non-shared environment (Liang & Eley, 2005).

Figure 4.1. Histogram of the distribution of MZ difference scores on sleep quality



Note. Figure taken from Barclay et al. (in press)

Table 4.4. Means, standard deviations (SD), and monozygotic (MZ) twin correlations for all study variables

	No. of pairs	Mean	SD	MZ correlation
Sleep Quality	180	5.48	2.86	.40**
Dependent Negative Life Events	185	1.17	1.46	.40**
Independent Negative Life Events	186	.60	.89	.52**
Education	185	5.02	1.66	.44**
Employment	171	.49	.50	.52**
Relationship /Single	183	.51	.50	.34**
Relationship Satisfaction	65	9.35	3.29	.05
Relationship Cohesion	64	9.26	2.89	.32*
Closest Friendship Quality	182	67.17	17.02	.26**
Friendship Quality	186	7.28	2.02	.26**
Deviant Peers - EYSTC	186	5.52	2.44	.35**
Deviant Peer Affiliations	186	5.15	2.67	.65**
Health	188	2.20	0.93	.34**

Note. *$p<.05$, **$p<.01$.

Table 4.5 displays the phenotypic correlations (Pearson's) between all study variables. The largest associations with sleep quality were with general health and dependent negative life events (r = .40 and .35, respectively). The results of the multiple univariate linear regression analyses are shown in **Table 4.6** for the total sample as well as for males and females separately. Column 'a' shows the results on absolute sleep quality scores. For males, the beta weights (β) were generally smaller for many of the

environmental measures, and there were fewer significant associations between the environmental measures and absolute sleep quality score, than for females. This suggests that a greater range of environmental factors are associated with sleep disturbance in females. Dependent negative life events, deviant peers and general health were correlates of sleep quality for both sexes. In addition, however, relationship satisfaction as well as friendship qualities were among the strongest correlates of sleep quality in females.

Column 'b' shows the results of the MZ differences analyses, which control for the influence of both genetic and shared environmental effects, using twin difference scores on the environmental measures to correlate with twin differences in sleep quality. In these analyses only general health remained significantly associated with sleep in males, and only relationship satisfaction in females. Thus, greater disparity in general health and relationship satisfaction for males and females respectively, is associated with increased differences in sleep quality between twins within a pair. These associations are entirely due to the non-shared environment and not due to genetic/ shared environmental factors. This is also indicated by the smaller β values for these variables in the analysis without controlling for genetic and shared environmental factors, compared with when these factors are controlled.

Table 4.5. Pearson's correlations between study variables

	Sleep Quality	Dep. Neg. Life Events	Ind. Neg. Life Events	Education	Relationship		Friendships		Deviancy		Health
					Satisfaction	Cohesion	Closest Friendship	Friendship Quality	Deviant Peers	Affiliations	
Sleep Quality	1										
Dep. Neg. Life Events	.35**	1									
Ind. Neg. Life Events	.12*	.27**	1								
Education	.00	.02	-.06	1							
Relationship Satisfaction	.19**	.15*	.12	-.08	1						
Relationship Cohesion	.18**	.13	-.07	-.03	.37**	1					
Closest Friendship Quality	-.18**	-.02	.08	.03	-.15*	-.27**	1				
Friendship Quality	.19**	.15**	-.02	-.02	.07	.11	-.40**	1			
Deviant Peers	.19**	.33**	.06	-.08	.15*	.23**	.17**	.01**	1		
Deviant Peer Affiliations	.03	.11*	-.03	-.07	.13	.14*	-.05	.14**	.23**	1	
Health	.40**	.40**	.20**	-.01	.09	.17*	-.13*	.10	.19**	.00	1

Note. *p<.05, **p<.01. Sleep quality: higher scores = poorer sleep quality; Dep./Ind. Neg. Life Events: higher scores = more life events experienced; Relationship satisfaction/ cohesion: higher scores = less satisfaction/cohesion; Closest friendship quality: higher scores = better friendship quality; Friendship quality: higher scores = more problems with friends; Deviant peers: higher scores = friends behave more deviantly; Deviant peer affiliations: higher scores = greater affiliation with deviant peers; Education: higher scores = higher level of achievement. Two variables were not included in the correlational analyses as they are binary variables: Employment status coded as 0 = in employment, 1 = full-time student (Mean PSQI scores = 5.28[SD = 2.85]; and 5.61[SD = 2.80], respectively, $t(347) = -1.12$, p=.26); and Relationship status coded as 0 = single, and 1 = in a relationship (Mean PSQI scores = 5.45[SD = 2.75]; and 5.49[SD = 2.89], respectively, $t(361) = -.12$, p=.90).

Table 4.6. Univariate linear regression analyses predicting (a) absolute sleep quality scores and (b) MZ difference scores to control for genetic vulnerability and shared environment, from the environmental measures

	(a) Absolute measures analysis			(b) MZ difference analysis		
	R^2	β	t	R^2	β	t
Dep. Negative Life Events						
Total	.13	1.02	**5.99****	.01	.37	1.59
Males	.13	1.18	**3.20****	.01	.26	.48
Females	.15	.96	**5.24*****	.02	.37	1.49
Ind. Negative Life Events						
Total	.02	.36	1.74	.00	.13	.68
Males	.04	.28	.77	.01	-.17	-.30
Females	.03	.42	**2.10***	.01	.20	1.25
Education						
Total	.00	-.01	-.05	.00	.09	.38
Males	.03	-.01	-.06	.01	.16	.41
Females	.01	-.04	-.20	.00	.03	.09
Employment status						
Total	.01	.17	1.02	.01	.25	.92
Males	.07	.65	**2.39***	.05	.71	1.32
Females	01	-.10	-.52	.01	-.02	-.06
Relationship/single						
Total	.00	.04	.22	.00	-.15	-.61
Males	.03	-.17	-.61	.02	-.37	-1.09
Females	.01	.18	.99	.01	.04	.12
Relationship Satisfaction						
Total	.04	.58	**2.79****	.06	.59	1.14
Males	.01	.16	.52	.05	-.08	-.08
Females	.08	.91	**3.88*****	.14	1.01	**2.40***

Table 4.6 (continued). Univariate linear regression analyses predicting (a) absolute sleep quality scores and (b) MZ difference scores to control for genetic vulnerability and shared environment, from the environmental measures

	(a) Absolute measures analysis			(b) MZ difference analysis		
	R^2	β	t	R^2	β	t
Relationship Cohesion						
Total	.03	.51	**2.32***	.03	.21	.43
Males	.06	.61	1.47	.05	.08	.06
Females	.03	.46	1.82	.04	.16	.29
Best Friendship Quality						
Total	.03	-.51	**-2.95***	.01	-.34	-1.45
Males	.04	-.34	-1.15	.01	-.17	-.35
Females	.05	-.58	**-2.86****	.03	-.42	-1.60
Friendship Quality						
Total	.04	.55	**2.75****	.02	.39	1.56
Males	.06	.50	1.67	.05	.62	1.45
Females	.04	.55	**2.25***	.01	.23	.81
Deviant Peers						
Total	.03	.53	**3.02****	.00	.10	.44
Males	.06	.48	**2.01***	.01	-.02	-.04
Females	.04	.62	**2.66****	.01	.06	.22
Deviant Peer Affiliations						
Total	.00	.04	.25	.00	.05	.18
Males	.03	.11	.38	.02	.26	.65
Females	.01	-.04	-.19	.01	-.07	-.26
Health						
Total	.17	1.17	**7.13****	.06	.80	**3.55****
Males	.16	1.15	**4.17*****	.20	1.56	**4.65*****
Females	.19	1.16	**5.79*****	.02	.34	1.37

Note. MZ = monozygotic. *p<.05, **p<.01, ***p<.001.

4.5 Discussion

The aim of this chapter was to address the second set of research questions posed in the introduction of this thesis. Specifically, the present study assessed (i) the associations between sleep quality and a range of candidate non-shared environmental factors; and (ii) whether there is a purely non-shared environmental component to these associations, i.e. whether these associations remain significant even after controlling for genetic and shared environmental factors. In order to achieve the second aim, the MZ twin differences design was used. When genetic and shared environmental factors were controlled, general health was significantly associated with sleep quality in males, whilst in females relationship satisfaction was significant. These findings suggest that it is the non-shared aspects of these environmental influences that are associated with sleep quality. All other environmental measures investigated did not remain significantly associated with sleep quality when genetic and shared environmental factors were controlled, which suggests that their associations with sleep quality are in part dependent on genetics and/or the shared environment. This study is to the authors' knowledge the first investigation of purely non-shared environmental components to the associations between the environment and sleep quality. Although a number of twin studies (including the results of Chapter 3) investigating the relative contribution of genetic and environmental influences on subjective sleep quality have shown that a large proportion of the variance is accounted for by the non-shared environment (see Heath, et al., 1990; Partinen, et al., 1983), the present analyses allow us to examine specific non-shared environmental influences. By selecting candidate environmental influences known to be associated with sleep quality, the results of this chapter allow us to further understand the possible mechanisms by which these associations occur. Further discussion of the main findings of this study is presented below, followed by an outline of the limitations specific to this study.

4.5.1 Associations between sleep and the environment

The majority of the environmental measures included in the present study (with the exception of deviant peer affiliations and education) were significantly associated with sleep quality. However, when assessing these associations independently by sex, it appears that some of these associations are sex-specific. For females, there appears to be greater associations and a wider range of environmental factors associated with sleep quality than males. Previous studies have demonstrated that sleep problems are more prevalent in women (Ohayon, 1997, 2005), and this could be in part due to the larger body of correlates of sleep disturbance in women as demonstrated here. One of the strongest associations with sleep quality for both males and females in the present study was with dependent negative life events. This concurs with previous research which has demonstrated the negative effects of stressful or negative life events on sleep (Lavie, 2001; Mezick, et al., 2009; Sadeh, 1996; Vahtera, et al., 2007). The finding that the association between sleep and life events was greater for dependent as compared to independent life events confirms the hypothesis that different types of life event are associated with sleep differentially. Although the cross-sectional nature of the current analyses meant that it was not possible to delineate cause and effect, one tentative explanation could be that feelings of responsibility involved in creating dependent negative life events could hinder sleep through the worry and cognitive rumination of the negative events to a greater extent than do independent negative life events. It is possible that an individual will ruminate more over a dependent negative life event, thinking of the ways in which the event could have been avoided, than independent life events for which they have no control.

However, the converse may also be true – that experiencing poor sleep quality leads one to experience more dependent negative life events. It is possible that experiencing poor sleep quality disrupts executive functioning in the prefrontal cortex

which consequently interferes with decision making and influences the experience of dependent negative life events. For example, induced sleep deprivation studies have demonstrated that daytime sleepiness disrupts executive functioning and consequently, decision making processes (Killgore, Balkin, & Wesensten, 2006); is associated with impaired cognitive and motor speed and higher cognitive abilities (Goel, Rao, Durmer, & Dinges, 2009); increases the risk of human-error related accidents (Dinges, 1995); and experimentally restricted sleep time affects behavioural alertness (Dinges & Rogers, 2005). It is possible that the stronger association between poor sleep quality and dependent negative life events as compared to independent negative life events reflects the fact that impaired sleep has consequences for cognitive functioning and decision making – processes involved in all of one's dependent experiences.

It is possible that the relationship between dependent negative life events and sleep may be accounted for by gene-environment correlation. That is, genetic factors on sleep quality may, either directly or via intermediate variables, influence exposure to negative life events. For example, a genetic predisposition to poor sleep quality may indirectly influence exposure to negative life events if an individual's behaviour is adversely affected by the consequences of a poor night's sleep. The present study lends some support for the possible presence of gene-environment correlation by showing that, when genetic and shared environmental factors are controlled, dependent negative life events no longer influence sleep quality. Explicit examination of the presence of gene-environment correlation (whilst accounting for the shared environment) is necessary before the presence of gene-environment correlation can be confirmed. This is because, as the MZ differences design is unable to tease apart the influence of genetics and the shared environment (as they are both entirely shared between MZ twins), there remains the possibility that the shared environment mediates the association between the non-shared environment and sleep quality. The following

chapter (Chapter 5) addresses this issue by explicitly investigating the presence of gene-environment correlation between negative life events and sleep quality in a sophisticated gene-environment interaction model.

Gene-environment correlation is also a possibility for the remaining environmental measures assessed here (with the exception of general health and relationship satisfaction), where associations with sleep quality reduced to non-significance when genetic and shared environmental factors were controlled. This means that influences that we would traditionally consider 'environmental' are in fact not working in a non-shared environmental way, but in part depend on genetics and/or the shared environment. This highlights the possible importance of our genetic and/or shared environment in selecting our unique environmental experiences.

4.5.2 Specific non-shared environmental influence: Health

Poorer general health was significantly associated with poorer sleep quality for both males and females. This is not surprising given that extensive research has linked sleep to a number of health problems (for example, see Briones, et al., 1996; Finn, et al., 1998; Gangswisch, et al., 2010; Roth, 2007) and given the comorbidity between sleep disturbance and psychiatric disorders (Ford & Kamerow, 1989; Morin & Ware, 1996). What is interesting here, however, is that the mechanism of action between sleep and health appears to differ between men and women. When controlling for genetic factors and the effects of the shared environment, general health only remained a significant correlate of sleep quality in males, acting as a purely non-shared environmental factor. Thus it appears for males that health outcomes that are independent of genetic and shared environmental effects are associated with sleep disturbance. This could shed light on the specific types of health problems associated with sleep, by identifying those

that are not linked to underlying heredity or shared environment. A plausible candidate is that of health problems resulting from accidents. Accidents are a common cause of morbidity and mortality especially in males within this age group and are not genetically determined (Barker, Power, & Roberts, 1996; Blum & Nelson-Mmari, 2004) (although it is possible that some individuals may be genetically prone to experience more accidents than others, i.e. through greater risk taking behaviour). Although it is likely that health problems that are in part genetically determined also influence sleep disturbance in males, the effect size in the MZ differences analysis is somewhat higher than in the absolute measures analysis, which may indicate that genetic effects on the association between health and sleep in males are minimal. For females, however, it appears that the types of health problems associated with sleep may be those linked to genetic effects, and may work either directly or via genetically influenced mediators. That is, the association between sleep and health for females may be accounted for by gene-environment correlation.

4.5.3 Specific non-shared environmental influence: Relationship satisfaction

Poor relationship satisfaction was significantly associated with poor sleep quality in females. This finding fits well with other studies which have shown that women reporting greater marital satisfaction experienced fewer sleep disturbances than those less maritally happy (Troxel, et al., 2009). Of critical importance to the present study is specific consideration of the non-shared aspect of the environment. The differences in relationship satisfaction between twins within a pair were associated with twin discrepancy in sleep quality. If we interpret this finding as evidence of a purely non-shared environmental component to the association between relationship satisfaction and sleep quality, this could be explained by the fact that a large amount of

satisfaction in romantic relationships is dependent on the behaviour of the partner as well as the individual, something which may be considered independent of one's genetic or shared environmental background. I acknowledge, however, the possibility that twins' genetic make-up may have some influence on their partner selection (see Rushton & Bons, 2005). One line of work investigating sleep and relationships suggests that we should consider the 'social context' of sleep (Troxel, Robles, Hall, & Buysse, 2007). That is, since the majority of adults typically share a bed with their partner, we should consider sleep as a dyadic process. It is possible that sleep disturbance in one individual increases the risk of sleep problems in their partner. Indeed it has been found that women living with snorers report more insomnia symptoms than women living with non-snorers (Ulfberg, Carter, Talback, & Edling, 2000), and that such disturbances from sleep are associated with greater marital dissatisfaction and divorce (Cartwright & Knight, 1987).

Furthermore, it has been hypothesized that sleep is a vulnerable state and that optimal conditions for sleep occur when one can sufficiently down-regulate vigilance and alertness – a process that requires one to feel safe and secure both emotionally and physically (Troxel, et al., 2007). It has been suggested that, from an evolutionary perspective, women may rely on their larger, more dominant male partner to provide safety and protection. Thus, it is plausible that if a couple are experiencing relationship difficulties, the woman may not experience the safety and security necessary for optimal sleep (Troxel, 2010). Evolutionarily this may seem plausible, however, it may be just as likely that males require such feelings of safety and security emotionally for optimum sleep. Although these theories are compelling in the older samples typically studied in research on sleep and relationships, the fact that we found similar trends in our younger sample (only a few of whom were living with partners at the time of the study [n=24]) suggests that it is not only the immediate, proximal closeness of a relationship that

affects sleep, but that other aspects of relationships outside of the bedroom are also important. Women are more sensitive to negative aspects of their relationships than men, spend more time thinking about events in their relationships, and become more upset from arguments (Kiecolt-Glaser & Newton, 2001). Thus, it is possible that women consequently ruminate over their relationship issues, to the extent that this rumination disrupts their sleep. Indeed, excessive cognitive activity and affective load are poor conditions for sleep (Espie & Wicklow, 2001), which may partially explain the gender differences so consistently evident in relation to sleep disturbance, and also the sex-dependent effect of relationships found here. Of course it is also possible that poor sleep adversely affects relationships. Evidence suggests that sleep deprivation has adverse effects on interpersonal and emotional responsiveness (Kahn-Greene, Lipizzi, Conrad, Kamimori, & Killgore, 2006), which may directly affect the way in which one interacts with their partner.

4.5.4 Limitations

There are a number of limitations specific to this study that require consideration when interpreting these findings. First regards the use of self-report data, which may be affected by recall and rater bias (for a more comprehensive discussion of the use of self-report measures generally, see Chapter 8, section 8.3.3). Most relevant to the present study, the twin difference scores may be affected by reporter effects if one twin has a tendency to respond more optimistically than their co-twin. However, the responses to the different measures showed some degree of specificity, indicated by the less than perfect correlations between them, suggesting that reporter effects were not cause for concern. A second limitation regards the use of the negative life event measure and the possibility of floor effects. As this measure is simply a checklist of events, it is likely that the sample, being relatively young, had not experienced many of the events. Indeed,

the average number of events experienced for both dependent and independent negative life events was only around 1. It is possible that even greater associations with poor sleep quality would emerge in individuals experiencing a greater number of events. An additional consideration regarding the life events measure is the temporal disparity between this measure (which assesses number of events experienced in the past year) and the PSQI (which assesses sleep quality in the past month). It would thus be useful to determine whether the duration since the event affects sleep differentially. It is likely that concurrent life events or those occurring in the past month have a greater influence on sleep in the past month than do events which occurred 12 months prior. A third limitation regards the low reliability of the scale from the Edinburgh Study of Youth Transitions and Crime (ESYTC) to assess friendship quality. This is likely due to the small number of items included in the measure. Accordingly, the results from this measure should be interpreted with caution and further research should perhaps consider an alternative scale to measure friendship quality. A fourth limitation centres on the fact that the sample largely consisted of females (65.8%), and so where our results are significant for females but not males may be a reflection of the smaller sample size and consequent reduction in power for males as compared to females, rather than being evidence of a true sex effect. Replications of the current findings in larger samples are warranted before we can confidently draw conclusions as to the possibility of sex-dependent effects. An additional consideration related to the sample is that the participants were recruited from a population sample rather than a clinical sample of individuals with a clinically diagnosable sleep disorder. As such the findings should be interpreted in terms of poor sleep quality in the normal range rather than extrapolating the current findings to clinical sleep disorders. Further studies investigating specific non-shared environmental influences on insomnia are necessary to determine whether the effects found here can justifiably be extrapolated (if indeed insomnia can be

considered the extreme of poor sleep quality). A fifth limitation is that even though the MZ twin difference measure allows us to determine the contribution of the non-shared environment independent of genetics and shared environmental influences, this component of variance inevitably includes measurement error, and so this should be taken into consideration when interpreting the current findings. Finally, as it was only possible to use a cross-sectional design (due to there only being one wave of data on sleep) it was not possible to determine the direction of effects. Whilst it is likely that the associations between sleep and the environment are bi-directional, further research using longitudinal designs is necessary to specifically address this issue (further discussion of this issue is reserved for Chapter 8, section 8.6.2).

Regardless of these limitations and the direction of the associations between sleep and the environment the results of this chapter suggest that genetic influences may be implicated in a wide range of seemingly 'environmental' variables (with the exception of general health and relationship satisfaction). These findings may give insight into the mechanisms by which genes and environments affect sleep, and suggests that their influences may not be independent. It would be essential for future research to explicitly investigate whether the association between these environmental variables and sleep quality are indeed accounted for by gene-environment correlation (and not obscured by the effects of the shared environment), in order to further determine their mechanism of action. It is to this issue that we now turn in Chapter 5, which focuses on gene-environment interplay in relation to the association between dependent negative life events and sleep quality. Additionally, it would be beneficial for research to incorporate a longitudinal element to the investigation of sleep and the environment to gain an understanding of the direction of these associations, and to help us to further understand the variability in sleep observed in the population.

4.6 Acknowledgments

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Chapter 5: Dependent Negative Life Events and Sleep Quality: An Examination of Gene-Environment Interplay

5.1 Overview

Negative life events are associated with sleep disturbances. Assessing the mechanisms by which these associations occur is important for understanding the aetiology of sleep problems. Substantial research suggests that genetic factors partially influence negative life events – a finding suggestive of gene-environment correlation. Furthermore, negative life events have been found to significantly interact with genetic influences to bring about a host of psychological and behavioural phenotypes. This chapter specifically explores gene-environment interplay in the associations between dependent and independent negative life events and sleep quality. Poor sleep quality was more strongly associated with dependent as compared to independent negative life events (r = .34 and .15, respectively), consistent with the findings from Chapter 4. Dependent negative life events were found to be partially heritable with genetic influence accounting for 40% of the variance. There was substantial overlap in the genetic influences on the association between dependent negative life events and poor sleep quality (rD = $.63_{[.45-.83]}$), suggesting gene-environment correlation, whereas environmental overlap was small (rE = $.11_{[-.02-.25]}$). Genetic influences accounted for a large proportion of the association ($81\%_{[.58-1.04]}$) with the remaining co-variance due to non-shared environment ($19\%_{[-.04-.42]}$). Finally, genetic liability to sleep quality was not moderated by dependent negative life events, although there was a non-significant trend for dependent negative life events to moderate non-shared environmental influences unique to sleep quality. These findings suggest that genetic and environmental effects on sleep quality are not necessarily distinct but to some extent work in concert.

5.2 Introduction

Chapter 4 examined associations between a host of environmental measures and sleep quality. One of the main findings from this study was that associations between many seemingly 'environmental' variables and sleep quality were in fact dependent on genetics and/or the shared environment. This was indicated by the finding that, when the effects of genetics and the shared environment were partialled out, the associations between many environmental variables and sleep quality were reduced to non-significance. Although the MZ differences design allows us to tease apart the effects of the non-shared environment from genetics and the shared environment, the distinction between the latter two influences is obscured as these influences are both entirely shared between MZ twins. In order to determine whether the associations between sleep and the environment are indeed accounted for by gene-environment correlation, or shared environmental correlation, an explicit test of gene-environment interplay on the variance components of sleep quality is necessary.

Given that the association between sleep quality and dependent negative life events was one of the largest of all associations between sleep and the environmental variables investigated in Chapter 4, and given that extensive research has highlighted the importance of investigating the role of stressful or negative life events in the occurrence of numerous sleep problems (Brummett, Krystal, Ashley-Koch, et al., 2007; Gregory, Caspi, et al., 2006; Heath, et al., 1998; Sadeh, 1996; Sadeh, Keinan, & Daon, 2004), this chapter focuses on explicitly testing the presence of gene-environment interplay, including both gene-environment correlation and interaction, between sleep quality and dependent negative life events. Furthermore, the association between independent negative life events and sleep quality is investigated in order to determine whether the finding of a smaller association as compared to dependent negative life events is confirmed in a larger sample than tested in Chapter 4 (where only MZ twins

were used). Understanding more about gene-environment interplay with regards to the associations between these distinct types of negative life events and sleep quality is important because it may provide an insight into the possible mechanisms acting between sleep and the environment.

5.2.1 Gene-environment correlation

As described in Chapter 1 (section 1.6.4.1) and Chapter 4 (section 4.2) certain environmental influences, such as dependent negative life events, show some degree of genetic influence (Bolinskey, et al., 2004; Federenko, et al., 2006; Kendler, et al., 1993; Plomin, et al., 1990; Thapar & McGuffin, 1996), which suggests rGE. Given the associations between sleep and negative life events it is possible that the genes influencing poor sleep quality also influence exposure to high risk environments. Analysing genetic liability to both sleep disturbance and the environmental stressors associated with sleep disturbance allows the detection of rGE. Finding overlap in the genetic influences attributable to these traits provides useful information about their aetiology. Specifically, here it would suggest that environmental risk factors for poor sleep quality are, in part, genetically driven by the same genes as those influencing sleep quality.

5.2.2 Gene-environment interaction

In addition to the possibility of gene-environment correlation, as described in Chapter 1 (section 1.6.4.2) recent genetic research has highlighted the importance of assessing the *interaction* between genes and environments in understanding the occurrence of traits (for example, see Moffitt, et al., 2005; Rutter, Moffitt, & Caspi, 2006). Gene-environment interaction (GxE) can be described as the moderation of

genetic risk in the presence of an identified environmental stressor (see Chapter 1, section 1.6.4.2 for further detail). Finding that the magnitude of genetic influence varies as a function of a measured environmental variable may guide molecular genetic research aimed at identifying specific genes involved in the trait under study (as candidate genes could be preferentially investigated in individuals in high-risk environments). Despite a growth of research assessing GxE for a number of psychological and behavioural traits, there is a dearth of research focussing on GxE in relation to sleep quality. Furthermore, quantitative genetic studies of GxE in relation to sleep quality are non-existent. In a molecular genetic analysis, one study to date found that a polymorphism of the serotonin gene (*5HTTLPR*) is associated with poor sleep quality, but specifically only in individuals experiencing chronic stress - conceptualised in the study as caregiving (Brummett, Krystal, Ashley-Koch, et al., 2007). What is unclear, however, is whether exposure to other negative life events has a modifying effect on the genetic and environmental factors influencing sleep. Negative life events have been identified as significant environmental stressors which modify genetic risk for a number of psychological problems, such as depression and anxiety (Caspi, et al., 2003; Lau & Eley, 2008; Lau, Gregory, Goldwin, Pine, & Eley, 2007; Silberg, et al., 1999; Silberg, et al., 2001) and externalising behaviours (Button, Lau, Maughan, & Eley, 2008). Whether such environmental stressors as negative life events have a similar effect on sleep quality, over and above a gene-environment correlation effect, is in question.

5.2.3 GxE in the presence of rGE

In order to fully understand the mechanisms by which genetic and environmental factors influence poor sleep quality it is necessary to simultaneously

consider gene-environment correlation and interaction. This is because it is possible that a GxE effect may be falsely identified in the presence of rGE (Purcell, 2002). For example, observing a greater genetic effect on sleep quality in those experiencing greater environmental risk is consistent with GxE, yet such a pattern could simply reflect rGE – that individuals with a high risk genotype will be more prevalent in high risk environments (Rutter & Silberg, 2002). As such, joint examination of these effects is necessary to correctly discriminate between correlation and interaction.

5.2.4 Aims

Accordingly, this chapter addresses the third set of research questions of this thesis, and examines: (i) the phenotypic associations between dependent and independent negative life events and sleep quality, in order to ascertain whether those life events under which one has some control (dependent) are more strongly associated with sleep problems than those under which one has no control (independent); (ii) the extent to which genes and environments account for individual variation in dependent negative life events; (iii) the degree of overlap in the genetic and environmental influences accounting for the association between dependent negative life events and sleep quality, to provide support for rGE; and (iv) the extent to which genetic and/ or environmental influences on sleep quality are moderated by increasing exposure to dependent negative life events. As it is likely that the association between dependent negative life events and sleep quality will be partially explained by shared genes, the final model assessed GxE in the presence of rGE to control for the possibility that an interaction effect could be spuriously detected as a result of gene-environment correlation (Purcell, 2002).

5.3 Method

5.3.1 Participants

The participants for this study were from the G1219 sample, and data was used from wave 4, as outlined in Chapter 2 (section 2.10).

5.3.2 Measures

Sleep quality was assessed by the PSQI global score, as outlined in Chapter 3 (section 3.3.2). Dependent and independent negative life events were assessed by the 'List of Threatening Experiences' (Brugha, et al., 1985), and the 'Coddington Stressful Life Events Scale' (Coddington, 1984), as outlined in Chapter 4 (section 4.3.2.2).

5.3.3 Statistical analyses

First, the degree of associations between dependent and independent negative life events and sleep quality were assessed using intraclass correlations. Second, univariate genetic models (as described in Chapter 2, section 2.4) were run to determine the extent to which genetic and environmental influences accounted for the variation in dependent negative life events. Third, a bivariate correlated factors models as described in section 2.7 (which allow the influences on one trait [e.g. additive genetic] to correlate with those on another trait) were run in order to determine the degree of overlap in these sources of influence between dependent negative life events and sleep quality; and the extent to which genetic and environmental influences accounted for the association between them. This enables us to assess whether genetic and environmental influences are shared between dependent negative life events and sleep quality – which would suggest the presence of rGE. Note that an analysis of independent negative life events and sleep quality was not performed as the phenotypic correlation was considered too

small (r = .15) to be decomposed meaningfully into genetic and environmental influences. Finally, models of gene-environment interaction in the presence of gene-environment correlation between measures were run (more detail is provided below in section 5.3.3.2). In all genetic model fitting analyses, where appropriate (i.e. indicated by the pattern of twin correlations), additive (A) as well as non-additive (D) genetic effects were tested. Furthermore, quantitative, qualitative and scalar sex differences were tested in the univariate analyses (as described in section 2.8).

All analyses were carried out using Mx (Neale, 1997), as described in Chapter 2, section 2.6.1, and incorporated the weight variable described in section 2.10 to account for selection bias and attrition. Furthermore, prior to analysis the data were regressed for the effects of age and sex as described in Chapter 3, section 3.3.3.1.1.

5.3.3.1 Genetic model-fitting

In all genetic models (univariate, bivariate, gene-environment interaction) the effects of additive genetic (A), shared environmental (C) *or* non-additive genetic (D), and non-shared environmental (E) variance components were examined. Furthermore, nested models (where certain parameters [e.g. C] were dropped) were run in order to determine the significance of the variance components (as described in section 2.6.2). The exception here are the moderating terms which, when successively dropped, often cause model instability. The most parsimonious genetic models, and those which resulted in the best fit compared to the saturated models (as indicated by the AIC value and a non-significant decrement in fit compared to fuller models), were selected for interpretation.

5.3.3.2 Models of GxE in the presence of rGE

Models of gene-environment interaction in the presence of gene-environment correlation were run as joint examination of these effects is necessary to correctly discriminate between correlation and interaction (Purcell, 2002). This model incorporates the moderator variable (i.e. dependent negative life events) as a measured trait alongside sleep quality to assess the extent to which the overlap in the genetic and environmental influences between the variables is moderated by dependent negative life events.

The model is based on a Cholesky decomposition (see Neale & Cardon, 1992) in which the variance/covariance structure of the two traits (dependent negative life events and sleep quality) is partitioned into genetic (e.g. D) and environmental influences (e.g. E) that are unique to sleep quality (e.g. D_u, E_u) and those that are common to both sleep quality and dependent negative life events (e.g. D_c, E_c) (note that only D and E were included in this model as the results of the bivariate correlated factors model suggested this to be the best-fitting model, and so the model is described in terms of these parameters). Dependent negative life events, however, are entered in the model twice: as a dependent variable as described above, and as a moderator in which levels will influence the effect of each of the variance component paths (d and e) expressed as beta coefficients ($ß_yM$ and $ß_zM$) (of note, the moderator is standardized so that it is possible to interpret changes in the variance components as standard deviations from the mean of the moderator variable). The moderator effects are further partitioned into those unique to sleep quality ($ß_{yu}M$ and $ß_{zu}M$), and those common to dependent negative life events and sleep quality ($ß_{yc}M$ and $ß_{zc}M$). The variance components independent of moderator level are: d_u, e_u, d_c and e_c. The linear function ($d_c + ß_{yc}M$) explicitly models the genetic overlap between the traits, as well as the interaction of the moderator on this overlap, allowing for the analysis of G×E in the presence of rGE. The linear function ($d_u + ß_{yu}M$)

models the 'unique-to-sleep' genetic variance, as well as the interaction of the moderator on this effect (see **Figure 5.1**). Significance of the moderating terms is assessed by 95% confidence intervals (CI). The significance of the moderating term on the common genetic path ($d_c + ß_{yc}M$) indicates that genetic effects are involved in both correlation and interaction with the moderator. Significance of the moderating term on the unique genetic path ($d_u + ß_{yu}M$) indicates that different genetic factors are involved in correlation with the moderator and interaction with it. The same logic can be applied to the other variance components (i.e. E).

Using the following equations and the parameter estimates derived from the full DE GxE in the presence of rGE model it is possible to calculate the changes in variance components as a function of the moderator. For example, at zero standard deviations from the moderator (i.e. at the mean of the moderator), non-additive genetics is calculated as:

$$d = \quad (d_u + d_c) \tag{5.1}$$

In order to calculate values of *d* at increasing (or decreasing) levels of the moderator, $ß_yM$ is added accordingly. For example, at one standard deviation from the mean of the moderator:

$$d = \quad (d_u + d_c) \pm ß_yM \tag{5.2}$$

where $ß_yM$ includes both the common ($ß_{yc}M$) and unique ($ß_{yu}M$) moderating terms; and where ± is used to denote whether the moderator is added (at increasing levels of the moderator) or subtracted (at decreasing levels of the moderator). At further increasing (or decreasing) levels of the moderator, $ß_yM$ is multiplied according to how many standard deviations it is from the mean. For example, at 2 standard deviations from the mean of the moderator:

$$d = \quad (d_u + d_c) \pm 2*(ß_yM) \tag{5.3}$$

and so on. The same logic can be applied to the variance components and corresponding moderating terms for the non-shared environment. The resulting unstandardised variance components at different levels of the moderator can then be plotted on a graph to visually inspect the gene-environment interaction.

Of note, it is also possible to plot changes in variance components at varying levels of the moderator as proportions (thus standardised values) of the total phenotypic variance. First, the total standardised variance (Var(T)) is calculated as:

$$\text{Var(T)} = (d + ß_yM)^2 + (e + ß_zM)^2 \tag{5.4}$$

where *d* and *e* are calculated as $(d_u + d_c)$ and $(e_u + e_c)$, respectively; and $ß_y$M and $ß_z$M include both the common and unique moderating terms. The proportion of *d* can then be calculated as follows:

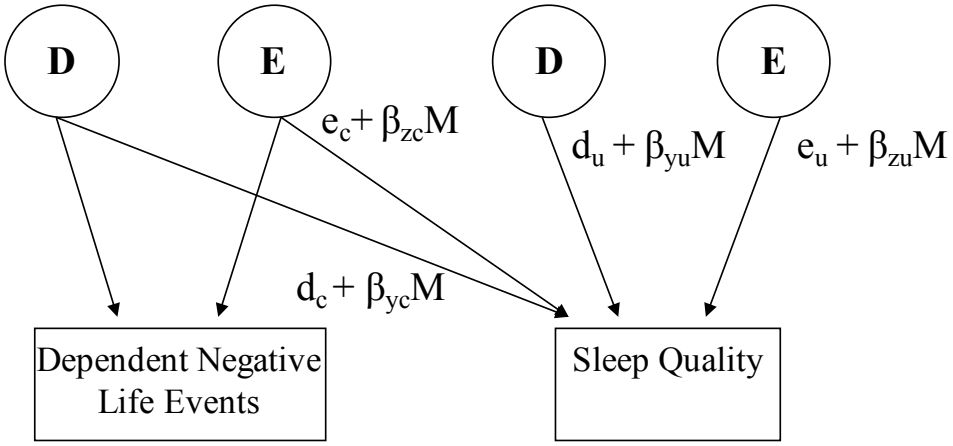
$$d = \quad \frac{(d + ß_yM)^2}{\text{Var(T)}} \tag{5.5}$$

and the proportion of *e* as:

$$e = \quad \frac{(e + ß_zM)^2}{\text{Var(T)}} \tag{5.6}$$

where $ß_y$M and $ß_z$M are multiplied in accordance with how many standard deviations they are from the mean. However, it is advised that the unstandardised estimates are interpreted, as presentation of the standardized estimates can be misleading (Purcell, 2002).

Figure 5.1. GxE interaction in the presence of gene-environment correlation.



Note. Figure adapted from Barclay et al. (2011). D = Additive genetic influence; and E = Non-shared environmental influence on sleep quality expressed as a linear function of the moderator by the inclusion of the coefficient terms for the variance components unique to sleep quality ($d_u + \beta_{yu}M$ and $e_u + \beta_{zu}M$) and those shared with dependent negative life events ($d_c + \beta_{yc}M$ and $e_c + \beta_{zc}M$). Figure is shown for one twin only.

5.4 Results

5.4.1 Descriptives

Table 5.1 displays means and standard deviations of raw scores for dependent and independent negative life events (descriptive statistics for global sleep quality is presented in Chapter 3, Table 3.3). There were significant sex differences in the means and standard deviations of dependent negative life events (change in fit of a model in which differences between the sexes were equated compared to a model where these estimates were free to vary between males and females: $\Delta\chi^2 = 13.40$, $\Delta df = 2$, $p<.01$), with males reporting significantly more life events than females; and in the standard deviations of independent negative life events ($\Delta\chi^2 = 13.71$, $\Delta df = 1$, $p<.01$), where there was significantly more variability in males. Of note, while the differences in number of negative life events experienced between the sexes were significant, the

effect sizes were small (d =.18 and d =.07 for dependent and independent negative life events, respectively). It is therefore unwise to place too much emphasis on this difference.

Table 5.1. Descriptive statistics. Means (standard deviations) of scores for dependent and independent negative life events

	Total	Males	Females	MZ	DZ	Sibs
DLE	1.22 (1.54)	1.39* (1.61)*	1.11* (1.48)*	1.18 (1.47)	1.22 (1.53)	1.29 (1.65)
ILE	.60 (.86)	.63 (.93)*	.57 (.80)*	.61 (.87)	.63 (.89)	.50 (.73)

Note. DLE = Dependent Negative Life Events (range = 0-13); ILE = Independent Negative Life Events (range = 0-8). Means and standard deviations of raw (untransformed) data. Sex differences for means and standard deviations were tested, * p <.01.

5.4.2 Phenotypic and twin correlations

The phenotypic correlations (presented in **Table 5.2**) indicated that experiencing more dependent and independent negative life events was significantly associated with poorer sleep quality. This effect was significantly stronger for dependent negative life events compared to independent negative life events (p<.05). The cross-twin within-trait correlations suggest that genetic influences are more important for dependent as opposed to independent negative life events. The cross-twin cross-trait correlations for MZ twins were more than double that of the corresponding DZ and sibling correlations for both the association between dependent negative life events and sleep quality, and independent negative life events and sleep quality, suggesting a role for non-additive genetic influences in explaining the associations between traits. However, as the phenotypic association between independent negative life events and sleep quality was

small (r=.15) power to decompose the association into genetic and environmental influences was limited and so further analysis of this association was not undertaken.

Table 5.2. Phenotypic correlations for monozygotic twins (MZ), dizygotic twins (DZ) and siblings (Sibs) (with 95% confidence intervals)

	DLE-DLE	ILE-ILE	PSQI-DLE	PSQI-ILE
Within Twins /			.34	.15
			(.29 - .39)	(.09 - .20)
Cross Twins				
MZ	.40	.49	.28	.10
	(.27 - .51)	(.37 - .59)	(.19 - .36)	(.01 - .19)
DZ	.12	.30	.05	.04
	(.01 - .23)	(.20 - .40)	(-.03 - .14)	(-.03 - .12)
Sibs	.08	.30	.10	.02
	(-.10 - .25)	(.11 - .46)	(-.04 - .23)	(-.11 - .14)

Note. PSQI = Pittsburgh Sleep Quality Index; DLE = Dependent Negative Life Events; ILE = Independent Negative Life Events. The model was constrained where appropriate. For example, the twin correlations were constrained so that those of the randomly selected twin 1's were the same as the randomly selected twin 2's. All analyses were run on transformed (i.e. age and sex regressed) data and include a weight variable to account for initial selection bias and attrition.

5.4.3 Univariate genetic analysis of dependent negative life events

Fit statistics for the univariate genetic models of dependent negative life events are shown in **Table 5.3**. The table first displays the fit of the saturated (fully unconstrained) model. Subsequently, qualitative, quantitative and scalar sex differences are tested, as are the significance of C and D. A 'DE' model in which sex differences were equated was considered the best-fitting model as indicated by the value of AIC (note that although model 12 [DE model allowing for scalar sex differences] had the lowest AIC value, there was no significant decrement in model fit when dropping the

scalar term in model 17: $\Delta\chi^2 = 3.25$, $\Delta df = 1$, p=.07, and so being more parsimonious, model 17 was selected for interpretation). Non-additive genetic influence accounted for 43%$_{[.31-.53]}$, and non-shared environmental influence accounted for 57%$_{[.47-.69]}$ of the variance in dependent negative life events.

5.4.4 Bivariate genetic analysis

Fit statistics from the bivariate correlated factors models are provided in **Table 5.4.** Note that because 'CE' and 'E' models did not provide a good fit to the data in the univariate models for sleep quality and dependent negative life events, these models were not tested in the bivariate analysis. Furthermore, as there was no evidence for qualitative or scalar sex differences in the univariate analyses, these were also not tested in the bivariate genetic analyses. Although there was also no evidence for quantitative sex differences in the univariate analyses, it is standard practice to explore such effects in bivariate models. Accordingly, an exploratory investigation of this was tested in the bivariate analysis as it is possible that there may be differences between the sexes in the magnitude to which genetic and environmental influences account for the association between phenotypes.

The model providing the best fit according to the AIC value was a 'DE' model in which all sex differences were equated (model 9). In this model there was moderate overlap in the additive genetic influences between sleep quality and dependent negative life events (rD = .63$_{[.45-.83]}$). This is evidence for rGE. Overlap in the non-shared environmental influences, however, was small and non-significant (rE = .11$_{[-.02-.25]}$). Furthermore, genetic influences accounted for a substantial proportion of the co-variance between the traits (81%$_{[.58-1.04]}$), with the remaining 19%$_{[-.04-.42]}$ attributable to the non-shared environment.

Table 5.3. Fit statistics for univariate genetic model fitting analyses of dependent negative life events

Model	Model Fit		Fit relative to saturated model			
	-2LL	*df*	$\Delta\chi^2$	Δdf	*p*	AIC
1. Saturated	5770.09	1282				
2. ACE QSD + Qual. SD on A	5805.70	1299	35.60	17	.01	1.60
3. ACE QSD + Qual. SD on C	5807.30	1299	37.21	17	.00	3.21
4. ADE QSD + Qual. SD on A	5804.15	1299	34.06	17	.01	.06
5. ADE QSD + Qual. SD on D	5804.15	1299	34.06	17	.01	.06
6. ACE QSD	5807.30	1300	37.21	18	.00	1.21
7. ADE QSD	5804.24	1300	34.14	18	.01	-1.86
8. ACE SSD	5810.56	1302	40.47	20	.00	.47
9. ADE SSD	5807.57	1302	37.48	20	.01	-2.52
10. AE SSD	5810.56	1303	40.47	21	.01	-1.53
11. CE SSD	5821.34	1303	51.25	21	.00	9.25
12. DE SSD	5807.60	1303	37.51	21	.01	-4.49
13. ACE NSD	5814.06	1303	43.96	21	.00	1.96
14. ADE NSD	5810.83	1303	40.74	21	.01	-1.26
15. AE NSD	5814.06	1304	43.96	22	.00	-.04
16. CE NSD	5825.01	1304	54.92	22	.00	10.92
***17. DE NSD**	**5810.86**	**1304**	**40.76**	**22**	**.01**	**-3.24**
18. E NSD	5848.67	1305	78.57	23	.00	32.57

Note. * = Best-fitting model; QSD = Quantitative sex differences (magnitude of parameter estimates can vary between males and females); Qual. SD = Qualitative sex differences on A, C or D (genetic or shared environmental correlation between males and females); SSD = Scalar sex differences (variance differences between males and females); NSD = no sex differences; -2LL = -2*(log likelihood); df = degrees of freedom; $\Delta\chi^2$ and Δdf = change in chi-square statistic and corresponding degrees of freedom (computed as the difference in likelihood and df between each model and the saturated model); *p* = probability; AIC – Akaike's Information Criterion statistic (calculated as $\chi^2 - 2\Delta df$). All analyses focus on transformed variables. All estimates were obtained from Mx and incorporated a weight to account for initial selection bias and selective attrition.

Table 5.4. Fit statistics for bivariate correlated factors models for sleep quality and dependent negative life events

	Model Fit		Fit relative to saturated model			
Model	-2LL	*df*	$\Delta\chi^2$	Δdf	*p*	AIC
1. Saturated	12125.75	2525				
2. ACE SD	12218.82	2590	93.07	65	.01	-36.93
3. ADE SD	12214.06	2590	88.31	65	.03	-41.69
4. AE SD	12222.95	2595	97.20	70	.02	-42.80
5. DE SD	12218.76	2595	93.01	70	.03	-46.99
6. ACE NSD	12229.00	2596	103.25	71	.05	-38.75
7. ADE NSD	12223.92	2596	98.17	71	.02	-43.83
8. AE NSD	12229.00	2599	103.254	74	.01	-44.75
***9. DE NSD**	**12226.64**	**2599**	**100.891**	**74**	**.02**	**-47.11**

Note. * = Best-fitting model; SD = magnitude of parameter estimates can vary between males and females; NSD = no sex differences; Scalar = variances can vary between males and females; -2LL = -2*(log likelihood); df = degrees of freedom; $\Delta\chi^2$ and Δdf = change in chi-square statistic and corresponding degrees of freedom (computed as the difference in likelihood and df between each model and the saturated model; *p* = probability; AIC – Akaike's Information Criterion statistic (calculated as $\Delta\chi^2 - 2\Delta df$). All analyses focus on transformed variables. All estimates were obtained from Mx and incorporated a weight to account for initial selection bias and selective attrition.

5.4.5 Models of GxE in the presence of rGE

Interactions between variance components (D and E were tested as these components best described the data in the bivariate correlated factors models) and dependent negative life events were examined in the presence of genetic correlations between the moderator and sleep quality. Although successively dropping the

moderating terms from the model often results in model instability, it is possible to do this to give an indication of the significance of these parameters. The parameter estimates from the full and nested models of GxE in the presence of rGE are presented in **Table 5.5**. The 95% confidence intervals around the moderating terms in the full model showed all moderating terms to be non-significant, however, dropping all these terms from the 'DE' model resulted in a significant worsening of fit (model 7: $\Delta\chi^2$ = 22.74, Δdf = 4, p<.001). The moderating term on the non-shared environmental influences unique to sleep quality (β_{zu}) seems to carry most of this effect, since dropping this term only resulted in a near significant worsening of fit (model 5: $\Delta\chi^2$ = 3.26, Δdf = 1, p =.07), whereas independently dropping the other moderating terms did not (all p's >.05). This suggests possible evidence for an environment-environment interaction (ExE), meaning that the environmental influences contributing to rGE and GxE may be distinct (however, given that the non-shared environmental path was not significant in the model including all moderating terms this finding should be interpreted with caution). Using the equations described in section 5.3.3.2, the parameter estimates from the fullest model (model 1) were used to calculate changes in the unstandardised variance components across levels of the moderator. These values are presented in **Table 5.6.** The resulting changes in the unstandardised variance components are plotted in **Figure 5.2**. For example, at 3 standard deviations from the mean number of dependent negative life events, d is calculated as:

$$(1.40 + 1.46) + 3*(-.26 + .21) = 2.71 \quad (5.7)$$

Table 5.5. Model fitting information and parameter estimates (with 95% confidence intervals) for the moderating terms from the models of GxE in the presence of rGE

	Parameter Estimates for Moderating terms				Fit Compared to Full DE Model		
Model	$ß_{xc}M$	$ß_{xu}M$	$ß_{zc}M$	$ß_{zu}M$	-2LL(df)	$\Delta\chi^2$ (Δdf)	*p*
1. Full DE	-.26 (-.56 - .06)	.21 (-.24 - .60)	.09 (-.19 - .34)	.25 (-.02 - .51)	8434.26 (2393)		
2. Drop $ß_{yc}M$	/	.13 (-.31 - .51)	-.09 (-.26 - .06)	.23 (-.03 - .48)	8436.86 (2394)	2.60 (1)	.11
3. Drop $ß_{yu}M$	-.23 (-.53 - .09)	/	.05 (-.21 - .31)	.35 (.18 - .52)	8435.16 (2394)	.90 (1)	.34
4. Drop $ß_{zc}M$	-.18 (-.37 - .01)	.17 (-.27 - .55)	/	.25 -.02 - .51)	8434.67 (2394)	.41 (1)	.52
5. Drop $ßz_{u}M$	-.24 (-.54 - .08)	.46 (.23 - .73)	.09 (-.17 - .33)	/	8437.52 (2394)	3.26 (1)	.07
6. Drop $ß_{yc}M$, $ß_{yu}M$, $ß_{zc}M$	/	/	/	.30 (.16 - .46)	8439.60 (2396)	4.80 (3)	.19
7. Drop all moderator terms	/	/	/	/	8457.00 (2397)	22.74 (4)	.00

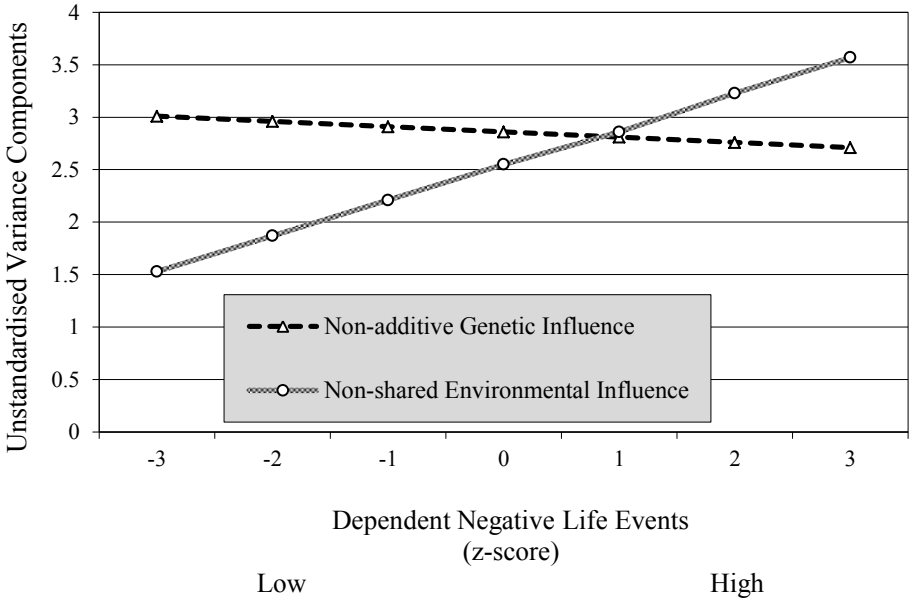
Note. $ß_{yc}M$, $ß_{yu}M$, $ß_{zc}M$, and $ß_{zc}M$ represent the moderating terms on the common non-additive genetic, unique non-additive genetic, common non-shared environmental, and unique non-shared environmental paths, respectively. -2LL = -2*(log likelihood); df = degrees of freedom; $\Delta\chi^2$ and Δdf = change in chi-square statistic and corresponding degrees of freedom; *p* = probability.

Table 5.6. An E x E interaction: Non-additive genetic and nonshared environmental variance on sleep quality tabulated against different values of the moderating variable (dependent negative life events)

Standard deviation from mean of moderator	d	e
-3	3.01	1.53
-2	2.96	1.87
-1	2.91	2.21
0	2.86	2.55
1	2.81	2.86
2	2.76	3.23
3	2.71	3.57

Note. d = unstandardised non-additive genetic variance; e unstandardised non-shared environmental variance. Unstandardised parameter values for variance components and moderator terms taken from the full 'DE' model are d_c = 1.40; d_u = 1.46; e_c = .32; e_u = 2.23 and $ß_{yc}$ = -.26; $ß_{yu}$ = .21; $ß_{zc}$ = .09; $ß_{zu}$ = .25. The moderator (dependent negative life events) is standardised with a mean = 0 and standard deviation 1.

Figure 5.2. Plot of total unstandardised variance components for sleep quality across levels of standardised dependent negative life events.



Note. Figure adapted from Barclay et al. (2011)

5.5 Discussion

The aim of this chapter was to address the third aim of this thesis – to examine the presence of statistical gene-environment interplay between sleep quality and negative life events. There were several noteworthy findings from the present study. First, the association between dependent negative life events and sleep quality was greater than that between independent negative life events and sleep quality, suggesting that different types of negative life events influence sleep differentially. This is in accordance with the findings from Chapter 4, but confirms this in a much larger sample (i.e. using MZ and DZ twins as well as siblings). Second, consistent with previous findings (for example, Bolinskey, et al., 2004; Federenko, et al., 2006; Kendler, et al.,

1993; Plomin, et al., 1990; Thapar & McGuffin, 1996), dependent negative life events were significantly influenced by genetic factors with no evidence for the shared environment. This latter finding clarifies the results of Chapter 4 by allowing us to tease apart the effects of genetics and the shared environment, and rule out the possibility of shared environment-environment correlation. Thus, the relationship between negative life events and sleep appears to depend to some extent on genetic variability. Third, there was substantial overlap in the genes influencing poor sleep quality and those influencing dependent negative life events, confirming the presence of gene-environment correlation. Finally, it was found that dependent negative life events did not moderate genetic liability to sleep quality. These findings are discussed in more detail below followed by a discussion of the limitations specific to this study.

5.5.1 Genetic influences on dependent negative life events and the association with sleep quality

It has been suggested that genetic factors could account for the trait-like stability of differential neuro-behavioural responding to sleep restriction (Banks & Dinges, 2007). In the context of the present study, this would suggest that the daytime consequences of a poor night's sleep may be genetically influenced. This is consistent with the finding presented here that dependent negative life events were partially influenced by genetic factors with no evidence of the shared environment. What is interesting here is that, in the bivariate analyses, the genetic factors influencing sleep quality were substantially shared with those influencing dependent negative life events. This suggests that one's genotype (i.e. a predisposition to poor sleep quality) increases exposure to high risk environments – evidence of rGE. Although this may suggest a direct pathway from genotype to experience, it is possible that the pathway by which

this gene-environment correlation takes effect is mediated by intermediate variables. For example, sleep disturbances are known to be associated with mood disorders such as anxiety and depression (Ford & Kamerow, 1989; Morin & Ware, 1996) - both of which are known to be associated with the experience of negative life events (Kendler & Karkowski-Shuman, 1997; Lau & Eley, 2008; Silberg, et al., 1999; Silberg, et al., 2001). Furthermore, results from other studies, including a paper from the G1219 study, have found that the associations between sleep, anxiety and depression are partially explained by shared genes (Gregory, Buysse, et al., 2011; Gregory, Rijsdijk, Dahl, McGuffin, & Eley, 2006). Thus, it is possible that rather than via a direct pathway, the genes that influence sleep are shared with those influencing anxiety and depression which, by their own nature, influence exposure to negative life events. Further exploration of the links between sleep and psychopathology is necessary to understand the mechanisms by which these variables are associated with negative life events, and longitudinal designs will enable us to determine the direction of the pathway between sleep and dependent negative life events.

5.5.2 Moderating effect of dependent negative life events on the genetic and environmental influences on sleep quality

There was no support for moderation of genetic effects on sleep quality by dependent negative life events. However, there was some evidence that non-shared environmental influences on sleep quality may be moderated by exposure to dependent negative life events. This would suggest that as one experiences more dependent negative life events, the non-shared environmental factors unique to sleep quality may increase in importance. In relative terms, this would indicate that genetic influences on sleep quality become less important at greater levels of negative life events. It is

possible that as one experiences more dependent negative life events, one may sleep poorly through the worry and anxiety caused by the events – which may act as a non-shared environmental factor. However, too much weight should not be placed on this finding as when using a more conservative approach (interpreting the full model) the effect fell short of significance. Although the findings suggest that genetic factors on sleep quality in the normal range are not influenced by the experience of negative life events, this may not be the case in extreme populations. Indeed, the frequency of cases scoring high on the extremes of negative life events was very low, and so a significant gene-environment interaction may not have been detected for this reason. As such, power to detect an interaction based on this variable alone was limited. Replicating this study in a selected group of poor sleepers and those with more extreme scores on negative life events will provide a more thorough test of the presence of gene-environment interaction in this context.

5.5.3 Limitations

There are several limitations specific to this study, the first regarding measurement. Self-report measures were used to assess both negative life events and sleep quality (as the issue regarding measurement of sleep quality is relevant to other chapters in this thesis, discussion of this limitation is reserved for Chapter 8, section 8.3.3). Self-report measures may be criticised on the grounds that they may not be reliable (relying on the subjective response and recall of the participant). Furthermore, it has been suggested that such self-reports of environmental measures may be measuring the individuals perception of the factor rather than the factor itself (Vinkhuyzen, van der Sluis, de Geus, Boomsma, & Posthuma, 2010). Kendler and Baker (2007) assessed the heritability of various environmental measures and found that estimates varied

according to rating method (e.g. self-report, external rater, videotape observation). However, the large-scale nature of the present study meant that obtaining more in depth measures of life events was not feasible. Despite this, the checklist nature of the negative life events measure meant that the respondents of this measure were less likely to suffer recall bias, since participants were simply required to indicate the presence or absence of a given event, and so the issue regarding the objectiveness of these ratings should not be cause for concern.

Another issue is that the distinction between dependent and independent negative life events was determined by considering the 'controllability' of the events. It is possible that the controllability could be perceived differently in individual cases. For example, the item 'death of a parent' was categorised as an independent, uncontrollable, event. In certain situations it is possible that an individual may feel partly responsible for such events (e.g. by causing stress to his/ her parents), and so the distinction between dependent vs. independent may be individual specific. Although a caveat of the present study, this method of assessment of dependent/ independent life events is standard and well accepted in the life event and depression literature (Brown & Harris, 1978; Cui & Vaillant, 1997; Rice, Harold, & Thapar, 2003; Silberg, et al., 2001; Williamson, Birmaher, Anderson, Alshabbout, & Ryan, 1995), and has been used in previous papers from the G1219 study (Lau & Eley, 2008; Liang & Eley, 2005). An additional consideration is that the distinction between dependent and independent negative life events based on the 'controllability' of the items may be confounded by the severity of the items included in the scales. For example, some items included in the 'independent' life events scale appear to be more severe than 'dependent' life events. To address this issue, 6 independent researchers were recruited and asked to rate the severity of the items included in both scales on a 7-point scale (1 = not very severe, to 7 = very severe) to determine whether there were systematic differences between them in

terms of severity. Although both measures contain items that are very severe and others that are less severe, the results suggested that there were systematic differences between the scales in terms of severity (dependent negative life events, average rating = 4.71, SD = .97; independent negative life events, average rating = 5.89, SD = .58, t = -2.57, p = .03). However, the severity of the items are not likely to explain the stronger associations between sleep and dependent as compared to independent life events (as the less severe scale – i.e. the dependent life events, showed particularly strong associations with sleep, perhaps contrary to what would be expected).

Overall, the present study suggests that the genetic and environmental influences on sleep quality are not entirely distinct, but work in concert via shared genes and intermediate variables. This should be considered in future research on the environmental origins of poor sleep quality. Further studies assessing gene-environment interaction and correlation between sleep quality and negative life events using measured genes will be essential in order to draw firm conclusions as to the presence/absence of this effect.

5.6 Acknowledgments

A manuscript based on the analyses of this chapter has been published and I thank my co-authors for their contribution.

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Chapter 6: Genetic and Environmental Influences on the Association between Sleep Quality and Diurnal Preference

6.1 Overview

One of the fundamental aims of this chapter is to understand how sleep quality is related to circadian rhythmicity. There are robust associations between sleep quality and intrinsic properties of the circadian system, yet little is known about what accounts for these associations. It is possible that a host of genetic and environmental influences work in concert to bring about these associations. Understanding the extent to which the genetic and environmental influences on sleep quality and diurnal preference (a marker of circadian rhythmicity) overlap will be informative in terms of identifying possible candidate genes involved in both phenotypes. The results from the present study indicated that a preference for eveningness was associated with poorer sleep quality ($r = .27_{[.22-.32]}$), and that this association was largely accounted for by genetic factors ($D = 96\%_{[.69-1.23]}$). Most importantly, there was substantial overlap in the genetic factors influencing sleep quality and diurnal preference ($rD = .52_{[.37-.70]}$); whereas overlap in the non-shared environmental factors between phenotypes was negligible ($rE = .02_{[-.11-.16]}$). The substantial overlap in the genetic influences between these phenotypes suggests that in order to identify candidate genes likely to be associated with sleep quality, researchers should focus on those that are implicated in diurnal preference, and vice-versa.

6.2 Introduction

Previous chapters have looked at the proportion of genetic and environmental influences on sleep quality; the specificity of the non-shared environment; as well as looking at the interplay between latent genetic and environmental factors on sleep quality. However, perhaps most important in the study of the aetiology of sleep quality is to identify the specific genes involved. In order to maximise the search for candidate genes associated with a trait, it is first of importance to use quantitative genetic designs to determine the possibility of shared genes between phenotypes. Bivariate quantitative genetic designs allow us to determine the degree of overlap in the genetic influences between two traits. If there is substantial genetic overlap between traits, genes already known to be associated with one phenotype may be worthy candidates for study in relation to other phenotypes with which it is associated. Minimal genetic overlap between phenotypes, however, would suggest that unique genes should be sought in relation to the phenotypes under study. Thus, by examining phenotypes which are theoretically related to the main phenotype under study, and using the knowledge of the genes associated with other phenotypes, it is possible to make inferences about possible candidate genes likely to be involved in the trait of interest. In light of this, this chapter investigates the relationship between sleep quality and another aspect of sleep which shows great inter-individual variability in the general population – diurnal preference.

6.2.1 Diurnal preference and its relationship to the circadian system and the regulation of sleep-wake behaviour

The regulation of sleep-wake behaviour is considered to be the product of two processes: the endogenous control of circadian rhythmicity and homeostatic regulation (Borbely, 1982; Borbely & Achermann, 1999; Daan, et al., 1984; Dijk & Lockley, 2002). The endogenous period of the circadian pacemaker is tightly constrained

between individuals, governed by many biological, psychological and environmental factors; however, circadian preferences and the entrainment of sleep-wakefulness to circadian rhythms show greater inter-individual variability (Kerkhof, 1985). Diurnal preference, otherwise termed 'chronotype', is considered to be the self-report analogue of circadian rhythm phase, and is often used as an indicator of these biological processes. Diurnal preference refers to an individual's preferred timing of sleep-wake activity and is measured on a continuum between two extremes from morningness to eveningness. 'Morning-types' so-called 'larks', find it easy to arise in the morning, function best at this time, and fall asleep easily during early evening. 'Evening-types', on the other hand, so-called 'owls', find it hard to get up early, are at their peak during late evening, and go to bed late, often in the early hours of the morning. Advanced and Delayed Sleep Phase Disorders (ASPD and DSPD, respectively) represent extremes of morning- and evening-type orientations (American Academy of Sleep Medicine, 2005), and are characterized by difficulty maintaining socially-normal sleep-wake hours even in the face of adverse social and occupational consequences. The majority of individuals in the general population (around 60-70%) lie around the midpoint of this continuum, reflecting an 'intermediate-type' morningness-eveningness disposition (Chelminski, Ferraro, Pertros, & Plaud, 1997; Natale & Cicogna, 2002).

A great deal of research has demonstrated that endogenous circadian rhythms differ between morning- and evening-types (Kerkhof & Van Dongen, 1996). Typically, the circadian rhythms of evening-types are phase delayed compared to morning-types, exhibiting later acrophases (peak times) in the daily fluctuations of many physiological variables, including core body temperature, heart rate, blood pressure, hormone secretion, and plasma melatonin secretion (Baehr, Revelle, & Eastman, 2000; Bailey & Heitkemper, 2001; Duffy, Dijk, Hall, & Czeisler, 1999; Taillard, Sanchez, Lemoine, & Mouret, 1990; Uusitalo, Ahonen, Gorski, Tuomisto, & Turjanmaa, 1988). Whatever the

likely mechanisms involved, it is these physiological differences in chronobiological phase that are thought to account for the observed differences in the preferred timing of sleep-wake activity in morning- and evening-types (Duffy, et al., 1999), and it is likely that genetic influences contribute to these processes.

6.2.2 Associations between diurnal preference, personality and sleep

In addition to investigating the physiological differences between morning- and evening-types, previous studies have investigated the personality dimensions and behavioural traits associated with circadian typology. Broadly speaking, in terms of the 'Big Five' personality factors (Costa & McCrae, 1992) a preference for morningness shows positive associations with agreeableness and conscientiousness, whereas a preference for eveningness shows positive associations with neuroticism and extraversion (as well as the subcomponent, impulsivity) (see Cavallera & Guidici, 2008 for a review; and Larsen, 1985; Randler, 2008b; Tonetti, Fabbri, & Natale, 2009). In one study conscientiousness appeared to be the single most important predictor of diurnal preference (Hogben, Ellis, Archer, & von Schantz, 2007). These personality dimensions have also been associated with sleep quality: conscientiousness being associated with good sleep quality and neuroticism with poor sleep quality (Gray & Watson, 2002). Behaviourally, evening-types compared to other chronotypes have more irregular daily lifestyle habits (Monk, Buysse, Potts, DeGrazia, & Kupfer, 2004); consume more psychoactive substances such as caffeine, alcohol and nicotine (Adan, 1994; Ishihara, et al., 1985); experience greater psychological distress, behavioural and emotional problems, in particular depression (Cavallera & Guidici, 2008; Chelminski, Ferraro, Pertros, & Plaud, 1999; Drennan, Klauber, Kiripke, & Goyette, 1991; Gaspar-Barba, et al., 2009; Hidalgo, et al., 2009; Lange & Randler, 2011); have lower self-control and elevated levels of procrastination (Digdon & Howell, 2008); have lower

overall satisfaction with life (Randler, 2008a); and hold dysfunctional beliefs about sleep (Ong, et al., 2007). Some of these difficulties have previously been associated with poor sleep quality, for example having an irregular lifestyle (Monk, Reynolds, Buysse, DeGrazia, & Kupfer, 2003); consuming alcohol and caffeine and smoking (see Ohayon, 2002 for a review); experiencing anxiety and depression (Gregory, Buysse, et al., 2011; Gregory, Rijsdijk, Dahl, et al., 2006); dissatisfaction with life (Paunio, et al., 2009), and holding dysfunctional beliefs about sleep (Edinger, Wohlgemuth, Radtke, Marsh, & Quillian, 2001; Gregory & O'Connor, 2002). It is possible that, since both diurnal preference and sleep quality have similar correlates, there may be more explicit links between them.

Perhaps more direct evidence for the associations between sleep quality and diurnal preference comes from noting that evening-types suffer from greater daytime sleepiness and dysfunction (Hidalgo, de Souza, Zanette, & Nunes, 2003; Vardar, et al., 2008), experience poorer sleep than morning-types (Megdal & Schernhammer, 2007; Ong, et al., 2007; Schnieder, et al., 2011; Selvi, et al., 2010; Shiihara, et al., 1998; Vardar, et al., 2008), are less alert after waking (Baehr, et al., 2000), and display other forms of sleep deficit, such as irregular sleep/wake habits (Giannotti, Cortesi, Sebastiani, & Ottaviano, 2002; Ishihara, Miyasita, Inugami, Fukuda, & Miyata, 1987; Taillard, et al., 1999). Furthermore, a relationship has been reported between the timing of an individual's biological clock and some types of chronic insomnia (Lack & Wright, 2007). When sleep is attempted at a time incongruent with one's biological clock, difficulties such as decreased total sleep time, impaired daytime functioning, cognitive impairment, fatigue, difficulty falling asleep, early morning awakenings, and chronic insomnia may arise - which may be seen not only in ASPD and DSPD, but also in conditions such as Shift Work Sleep Disorder (American Academy of Sleep Medicine, 2005). While there appears to be an association between sleep quality and diurnal

preference, what is currently unclear is what accounts for this association. One possibility is that shared genes are important in accounting for the association between these phenotypes.

6.2.3 Genetic and environmental influences on diurnal preference

Twin studies have determined that the timing of an individual's preferred sleep-wake cycle is to some extent under genetic control, accounting for roughly half of the variability in the phenotype (Drennan, Selby, Kripke, Kelsoe, & Gillin, 1992; Hur, et al., 1998; Koskenvuo, et al., 2007; Vink, et al., 2001); and numerous studies have investigated the molecular genetic basis for these inter-individual differences in the phase position of the biological 'clock' (Archer, et al., 2003; Archer, Viola, Kyriakopoulou, von Schantz, & Dijk, 2008; Carpen, Archer, Skene, Smits, & von Schantz, 2005; Carpen, et al., 2006; Dijk & Lockley, 2002; Katzenberg, et al., 1998; Lee, Paik, Kang, Lim, & Kim, 2007). Specifically, polymorphisms of 'clock' genes such as CLOCK and those of the PERIOD gene family have repeatedly been investigated in relation to circadian timing and preferences (more detailed information is reserved for Chapter 7). Furthermore, results of numerous studies, as well as those presented earlier in this thesis, provide substantial evidence for the influence of genetics on sleep quality (see Chapter 3 for further details). Given the well established relationship between sleep quality and diurnal preference it is worthwhile examining the extent to which genetic and environmental influences account for this association. Such an investigation may be useful in understanding why differences between chronotypes may be associated with sleep problems. Finding genetic/environmental overlap in the genes/environments influencing sleep quality and diurnal preference would suggest that once we have found genetic/environmental influences associated with one phenotype, the same genes/environments may be worth exploring as to their role in other

phenotypes with which it is associated - thus aiding the search for candidate genes underlying sleep disturbance.

6.2.4 Aims

Accordingly, this study addresses the fourth set of research questions outlined in Chapter 1 of this thesis, and investigates: (i) the extent to which genetic and environmental factors influence diurnal preference; (ii) the strength of the association between sleep quality and diurnal preference; (iii) the extent to which the genetic and environmental influences on diurnal preference overlap with those influencing sleep quality; and (iv) the extent to which the association between sleep quality and diurnal preference is accounted for by genetic and environmental influences.

6.3 Method

6.3.1 Participants

The participants for this study were from the G1219 sample, and data was used from wave 4, as outlined in Chapter 2 (section 2.10).

6.3.2 Measures

Sleep quality was assessed by the PSQI global score, as outlined in Chapter 3 (section 3.3.2). Diurnal preference was assessed by the Morningness-Eveningness Questionnaire (MEQ: Horne & Östberg, 1976), one of the most widely used measures for assessing diurnal preference. The MEQ was chosen over other instruments of circadian timing, such as the MCTQ (Roenneberg, et al., 2003), because the study aimed to investigate how individual *preference* for sleep-wake activity was influenced by genes and the environment, rather than determining the extent that *actual* sleep timing was influenced by these factors (as it is likely that actual bed-times are

influenced by the environment, the current investigation centred on whether preference for sleep timing is modified by these factors). The MEQ is a 19-item self-report questionnaire that assesses individual preference in the timing of daytime activities, sleeping habits, hours of peak performance, and times of 'feeling best' and maximum alertness. The items included in the MEQ, and the scoring methods for each item, are outlined in **Table 6.1**. The scores to each of the items are summed to give a total score on the morningness-eveningness dimension ranging from 16-86. Higher scores indicate greater 'morningness' and lower scores indicate greater 'eveningness'. However, for the present analyses the total MEQ scale was reversed so that a higher score indicated greater eveningness. This procedure was employed so that it was possible to decompose a positive correlation for ease of interpretation for the reader. The MEQ has previously been shown to have excellent psychometric properties with favourable content validity (Horne & Östberg, 1976) and an internal reliability coefficient (Cronbach's alpha, *a*) of .82 (Smith, Reilly, & Midkiff, 1989). In the present sample Cronbach's *a* = .76.

Table 6.1. Items included in the 'Morningness-Eveningness Questionnaire'

1. Considering your own "feeling-best" rhythm, at what time would you get up if you were entirely free to plan your day?				
1 = 11am-12pm	2 = 9:45-10:59am;	3 = 7:45-9:44am	4 = 6:30-7:44am	5 = 5-6:29am
2. Considering your own "feeling-best" rhythm, at what time would you go to bed if you were entirely free to plan your evening?				
1 = 1:45-3am	2 = 12:30-1:44am	3 = 10:15pm-12:29am	4 = 9-10:14pm	5 = 8-8:59pm
3. If there is a specific time at which you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?				
1 = very dependent	2 = fairly dependent	3 = slightly dependent	4 = not at all dependent	
4. Assuming adequate environmental conditions, how easy do you find getting up in the mornings?				
1 = not at all easy	2 = not very easy	3 = fairly easy	4 = very easy	

Table 6.1 (continued). Items included in the 'Morningness-Eveningness Questionnaire'

5. How alert do you feel during the first half-hour after having woken in the mornings?

1 = not at all alert	2 = slightly alert	3 = fairly alert	4 = very alert

6. How is your appetite during the first half-hour after having woken in the mornings?

1 = very poor	2 = fairly poor	4 = fairly good	4 = very good

7. During the first half-hour after having woken in the morning, how tired do you feel?

1 = very tired	2 = fairly tired	3 = fairly refreshed	4 = very refreshed

8. When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

1 = > 2 hours later	2 = 1-2 hours later	3 = < 1 hour later	4 = Seldom/never later

9. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 7am-8am. Bearing in mind nothing else but your own "feeling-best" rhythm, how do you think you would perform?

1 = would find it very difficult	2 = would find it difficult	3 = would be on reasonable form	4 = would be on good form

10. At what time in the evening do you feel tired and as a result in need of sleep?

1 = 2-3am	2 = 12:45-1:59am	3 = 11:30pm-12:44am	4 = 9-10:15pm	5 = 8-9pm

11. You wish to be at your peak performance for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day and considering only your own "feeling-best" rhythm which one of the four testing times would you choose?

0 = 7-9pm	2 = 3-5pm	4 = 11am-1pm	6 = 8am-10am

12. If you went to bed at 11pm at what level of tiredness would you be?

0 = not at all tired	2 = a little tired	3 = fairly tired	5 = very tired

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which one of the following events are you most likely to experience?

1 = will not wake up until later than usual	2 = will wake up at usual time but will fall asleep again	3 = will wake up at usual time and will dose thereafter	4 = will wake up at the usual time and will not fall asleep

14. One night you have to remain awake between 4am-6am in order to carry out a night watch. You have no commitments the next day. Which one of the following alternatives would suit you best?

1 = would not go to bed until watch was over	2 = would take a nap before and sleep after	3 = Would take a good sleep before and nap after	4 = would take all sleep before watch

Table 6.1 (continued). Items included in the 'Morningness-Eveningness Questionnaire'

15. You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your "feeling-best" rhythm which one of the following would you choose?

1 = 7-9pm	2 = 3-5pm	3 = 11-1am	4 = 8-10am

16. You have decided to engage in hard physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 10pm-11pm. Bearing in mind nothing else but you own "feeling-best" rhythm how well do you think you would perform?

1 = would be on good form	2 = would be on reasonable form	3 = would find it difficult	4 = would find it very difficult

17. Suppose that you can choose your own work hours. Assume that you worked a five hour day (including breaks) and that your job was interesting and paid by results. Which five consecutive hours would you select?

1 = 5pm-3am	2 = 2-4pm	3 = 9am-1pm	4 = 8-9pm	5 = 4-8pm

18. At what time of the day do you think that you reach your "feeling-best" peak?

1 = 10pm-4am	2 = 5-9pm	3 = 10am-4pm	4 = 8-9am	5 = 5-7am

19. One hears about "morning" and "evening" types of people. Which one of these types do you consider yourself to be?

0 = Definitely "evening" type	2 = rather more an "evening" than a "morning" type	4 = rather more a "morning" than an "evening" type	6 = definitely a "morning" type

In order to determine whether diurnal preference was associated with actual behaviour, scores on the MEQ were examined in relation to reported bed and arising times (these measures were taken from the PSQI and are typically used to calculate sleep duration). There was a significant association between diurnal preference (MEQ total score) and actual bedtimes, (r = .50, p<.01), indicating that greater eveningness preference was associated with going to bed later, and that greater morningness preference was associated with going to bed earlier. There was also a significant association between diurnal preference and getting-up time (r = .42, p<.01) indicating that greater eveningness was associated with later getting-up time, and greater morningness with earlier getting-up time. Of note it should be considered that actual

bedtimes and getting-up times may be influenced by many factors other than diurnal preference, such as school and work obligations (hence, the reason why a perfect correlation was not expected).

6.3.3 Statistical Analyses

First the degree of association between sleep quality and diurnal preference was assessed using intraclass correlations. Second, a series of univariate genetic models (as described in section 2.4) on MEQ were run in order to determine the extent to which genetic and environmental influences accounted for the variation in diurnal preference. Third, a bivariate correlated factors model (as described in section 2.7) was run in order to determine the extent to which the aetiological influences on diurnal preference overlapped with those on sleep quality; and the extent to which the association between sleep quality and diurnal preference was accounted for by genetic and environmental influences. In the univariate analysis quantitative, qualitative and scalar sex differences were tested (as described in section 2.8); whereas in the bivariate analyses only quantitative sex differences were tested, as is standard practice.

All analyses were carried out using Mx (Neale, 1997), as described in section 2.6.1, and incorporated the weight variable described in section 2.10 to account for selection bias and attrition. Furthermore, prior to analysis the data were regressed for the effects of age and sex as described in Chapter 3, section 3.3.3.1.1.

6.3.4.1 Genetic model-fitting

As in previous chapters, in all genetic models the effects of additive genetic (A), shared environmental (C) *or* non-additive genetic (D), and non-shared environmental (E) variance components were examined. Furthermore, nested models (where certain parameters [e.g. C] were dropped) were run in order to determine the significance of the

variance components (as described in section 2.6.2). The most parsimonious genetic models, and those which resulted in the best fit compared to the saturated models (as indicated by the AIC value and a non-significant decrement in fit compared to fuller models), were selected for interpretation.

6.4 Results

6.4.1 Descriptives

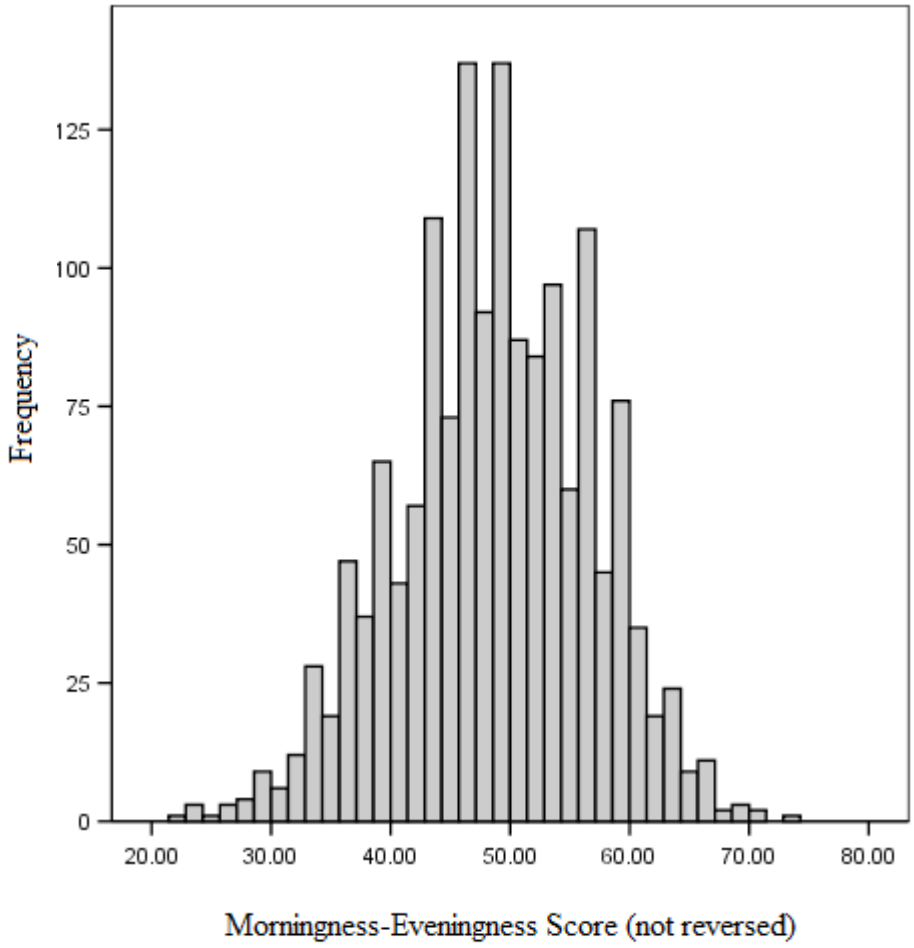
The frequencies of scores on the MEQ are displayed in **Figure 6.1.** Skew was not considered problematic for MEQ (MEQ skew = -.17, [SE = .09]), and so the variable was not transformed for this purpose. **Table 6.2** displays the means and standard deviations of raw scores on the morningness-eveningness questionnaire split by sex and zygosity (descriptive statistics for global sleep quality is presented in Chapter 3, Table 3.3). There were significant sex differences in diurnal preference (fit of model incorporating sex differences compared to fully constrained model: $\Delta\chi^2 = 29.61$, $\Delta df = 2$, $p<.01$), with males reporting slightly greater eveningness.

Table 6.2. Descriptive statistics. Means (standard deviations) of scores for dependent and independent negative life events

	Total	Males	Females	MZ	DZ	Sibs
MEQ	53.46	54.88	52.42	51.77	54.10	53.64
	(8.13)	(8.38)*	(7.78)*	(7.67)	(8.20)	(8.20)

Note. MEQ = Morningness-Eveningness Questionnaire (reversed so that higher scores indicate greater eveningness). Means and standard deviations of raw (untransformed) data. Sex differences for means and standard deviations were tested, * $p<.01$.

Figure 6.1. Histogram of the frequency of total MEQ scores



6.4.2 Phenotypic and twin correlations

Phenotypic and twin correlations are presented in **Table 6.3**. There was a significant phenotypic correlation between sleep quality and diurnal preference (r = .27), suggesting that greater eveningness preference is associated with poorer sleep quality (and conversely that greater morningness preference is associated with better sleep quality). The cross-twin within-trait correlations on diurnal preference suggest that non-additive genetic influences may be important (as indicated by the MZ correlations being more than twice that of the DZ and sibling correlations). Likewise, the cross-twin cross-trait correlations (e.g., the correlation between diurnal preference in twin 1 and

sleep quality in twin 2) suggest that non-additive genetic influences may be important for explaining the association between sleep quality and diurnal preference.

Table 6.3. Phenotypic correlations for monozygotic twins (MZ), dizygotic twins (DZ) and siblings (Sibs) (with 95% confidence intervals)

	MEQ-MEQ	PSQI-MEQ
Within Twins	/	.27 (.22 - .32)
Cross Twins		
MZ	.50 (.39 - .60)	.25 (.16 - .33)
DZ	.10 (.00 - .21)	.04 (-.04 - .12)
Sibs	.17 (.00 - .34)	.05 (-.08 - .18)

Note. PSQI = Pittsburgh Sleep Quality Index; MEQ = Morningness-Eveningness Questionnaire; MZ = monozygotic; DZ = dizygotic. All twin correlations were obtained from Mx incorporating a weight to account for selection bias and attrition. The model was constrained where appropriate. For example, the twin correlations were constrained so that those of the randomly selected twin 1's were the same as the randomly selected twin 2's. All analyses were run on transformed (i.e. age and sex regressed) data and include a weight variable to account for initial selection bias and attrition.

6.4.3 Univariate genetic analysis of diurnal preference

Fit statistics for the univariate genetic models of diurnal preference are shown in **Table 6.4**. The table first displays the fit of the saturated (fully unconstrained) model. Subsequently, qualitative, quantitative and scalar sex differences are tested, as are the significance of C and D. The best-fitting model was a 'DE' model which allowed for scalar sex differences (note that the scalar could not be dropped from the model as doing so resulted in a significant decrement in fit: fit of model 11 compared to model

16: $\Delta\chi^2$ = 4.64, Δdf = 1, p=.03). Accordingly, male variance was estimated as 9% greater than the female variance. Non-additive genetic influences accounted for 53%$_{[.19-.61]}$ and non-shared environmental influences accounted for 47%$_{[.39-.58]}$ of the variance in diurnal preference.

6.4.4 Bivariate genetic analysis

Fit statistics from the bivariate correlated factors models are provided in **Table 6.5.** Note that because 'CE' and 'E' models did not provide a good fit to the data in the univariate models for sleep quality and diurnal preference, these models were not tested in the bivariate analysis. Furthermore, as there was no evidence for qualitative or scalar sex differences (except for diurnal preference) in the univariate analyses for *both* traits, these were also not tested in the bivariate genetic analyses. A 'DE' model in which sex differences were equated (model 9) provided the best-fit to the data. From this model there was substantial overlap in the non-additive genetic influences between sleep quality and diurnal preference (rD = .52$_{[.37-.70]}$). There was negligible overlap in the non-shared environmental influences between these phenotypes (rE = .02$_{[-.11-.16]}$). The proportion of the phenotypic correlation accounted for by non-additive genetic and non-shared environmental influences was also estimated in this model. Overall, non-additive genetic influence accounted for 96%$_{[.69-1.23]}$ of the phenotypic association between sleep quality and diurnal preference; and non-shared environmental influence accounted for the remaining 4%$_{[-.23-.31]}$ of the covariance.

Table 6.4. Fit statistics for univariate genetic model fitting analyses of diurnal preference

	Model Fit		Fit relative to saturated model			
Model	-2LL	*df*	$\Delta\chi^2$	Δdf	*p*	AIC
1. Saturated	8380.523	1295				
2. ACE QSD + Qual. SD on A	8396.616	1312	16.09	17	.52	-17.91
3. ACE QSD + Qual. SD on C	8399.468	1312	18.95	17	.33	-15.06
4. ADE QSD + Qual. SD on A	8392.893	1312	12.37	17	.78	-21.63
5. ADE QSD + Qual. SD on D	8392.864	1312	12.34	17	.78	-21.66
6. ACE QSD	8399.468	1313	18.95	18	.40	-17.06
7. ADE QSD	8392.893	1313	12.37	18	.83	-23.63
8. ACE SSD	8402.337	1315	21.81	20	.35	-18.19
9. ADE SSD	8393.844	1315	13.32	20	.86	-26.68
10. AE SSD	8402.337	1316	21.81	21	.41	-20.19
***11. DE SSD**	**8393.844**	**1316**	**13.32**	**21**	**.90**	**-28.68**
12. ACE NSD	8407.421	1316	26.90	21	.17	-15.10
13. ADE NSD	8398.478	1316	17.96	21	.65	-24.05
14. AE NSD	8407.421	1317	26.90	22	.22	-17.10
15. CE NSD	8428.186	1317	47.66	22	.00	3.66
16. DE NSD	8398.478	1317	17.96	22	.71	-26.05
17. E NSD	8460.512	1318	79.99	23	.00	33.99

Note. * = Best-fitting model; QSD = Quantitative sex differences (magnitude of parameter estimates can vary between males and females); Qual. SD = Qualitative sex differences on A, C or D (genetic or shared environmental correlation between males and females); SSD = Scalar sex differences (variance differences between males and females); NSD = no sex differences; -2LL = -2*(log likelihood); df = degrees of freedom; $\Delta\chi^2$ and Δdf = change in chi-square statistic and corresponding degrees of freedom (computed as the difference in likelihood and df between each model and the saturated model); *p* = probability; AIC – Akaike's Information Criterion statistic (calculated as $\Delta\chi^2 - 2\Delta df$). All analyses focus on transformed variables. All estimates were obtained from Mx and incorporated a weight to account for initial selection bias and selective attrition.

Table 6.5. Fit statistics for bivariate correlated factors models for sleep quality and diurnal preference

	Model Fit		Fit relative to saturated model			
Model	-2LL	*df*	$\Delta\chi^2$	Δdf	*p*	AIC
1. Saturated	17698.226	2538				
2. ACE SD	17754.508	2603	56.28	65	.77	-73.72
3. ADE SD	17746.001	2603	47.78	65	.95	-82.23
4. AE SD	17764.788	2608	66.56	70	.59	-73.44
5. DE SD	17757.196	2608	58.97	70	.82	-81.03
6. ACE NSD	17770.576	2609	72.35	71	.43	-69.65
7. ADE NSD	17759.407	2609	61.18	71	.79	-80.82
8. AE NSD	17770.576	2612	72.35	74	.53	-75.65
***9. DE NSD**	**17761.940**	**2612**	**63.71**	**74**	**.80**	**-84.29**

Note. * = Best-fitting model; SD = magnitude of parameter estimates can vary between males and females; NSD = no sex differences; -2LL = -2*(log likelihood); df = degrees of freedom; $\Delta\chi^2$ and Δdf = change in chi-square statistic and corresponding degrees of freedom (computed as the difference in likelihood and df between each model and the saturated model); *p* = probability; AIC – Akaike's Information Criterion statistic (calculated as $\Delta\chi^2 - 2\Delta df$). All analyses focus on transformed variables. All estimates were obtained from Mx and incorporated a weight to account for initial selection bias and selective attrition.

6.5 Discussion

The principal aim of the present study was examine the extent to which the genetic and environmental influences on diurnal preference overlapped with those influencing sleep quality; and the extent to which these aetiological influences accounted for the association between the phenotypes. The main findings of this chapter are discussed below followed by an outline of the limitations specific to this chapter.

6.5.1 Distribution of diurnal preference scores

The overall mean diurnal preference score in the present study was as expected, reflecting an intermediate 'morningness-eveningness' disposition (note that this scale has been reversed – which should be taken into consideration when comparing this score with scores from other papers using the MEQ). This is in line with previous research which has suggested that the majority of individuals in the general adult population score somewhere around the midpoint on the morningness-eveningness continuum (Chelminski, et al., 1997; Horne & Östberg, 1976). In a study by Caci and colleagues (2009) which included 426 young adults aged between 17 and 46 years (Mean = 23 years; SD = 4.48 years), the average morningness-eveningness score was around 49 – almost identical to that of the present sample (mean score in present sample = 53 [reversed]; equating to a score of 49 [un-reversed]). Furthermore, Hogben and colleagues (2007) observed a mean score of 47 in a sample of 617 young adults aged between 18 and 39 years (mean age = 25 years; SD = 5.5 years).

Whilst the average score in the present sample appears to be in line with other research of young adults, a substantial amount of research has indicated that diurnal preference changes with age, and so the mean score in other age groups may be somewhat different. Several lines of evidence suggest a quadratic relationship between morningness-eveningness and age. For example, it is commonly observed that whilst children are often 'morning' oriented (Werner, LeBurgeois, Geiger, & Jenni, 2009), between the period of pre-adolescence to late adolescence individuals become more 'evening' oriented with a peak at around age 20 years (Gau & Soong, 2003; Hur, et al., 1998; Kerkhof, 1985; Kim, Dueker, Hasher, & Goldstein, 2002; Park, et al., 2002; Russo, Bruni, Lucidi, Ferri, & Violani, 2007; Shinkoda, Matsumoto, Park, & Nagashima, 2000). Yet beyond adolescence, it is typically observed that individuals become more 'morning' oriented throughout the lifespan (Carrier, et al., 1997; Kramer,

et al., 1999; Paine, Gander, & Travier, 2006; Park, et al., 2002; Robilliard, et al., 2002; Roenneberg, et al., 2007; Taillard, et al., 1999; Tonetti, Fabbri, & Natale, 2008). It appears that biological factors may account for the transition to eveningness from childhood to adolescence, with little influence of psychosocial factors (Carskadon, Vieira, & Acebo, 1993). Furthermore, the shift towards morningness from adolescence through adulthood is suggested to occur due to changes in the endocrine system, in particular the changes in the sexual hormone milieu during puberty and, in older women, the menopause (Randler & Bausback, 2010; Roenneberg, et al., 2007). Investigation of a specific gene (*PER3*) on diurnal preference has also indicated differential effects by age (Jones, et al., 2007). The robust evidence for an association between diurnal preference and age suggests that the investigation of the aetiological underpinnings of diurnal preference should consider the possibility that results may differ as a function of age. Since the participants in the present sample were all young adults (clustering around the age of 20 years), it was not possible to investigate the possibility of age-effects on diurnal preference, and so extrapolation of the present results should be confined to the age group under study.

In addition to age-related changes in diurnal preference, it is known that diurnal preference differs between males and females. Typically, women are more morning-oriented than men (Adan & Natale, 2002; Robilliard, et al., 2002; Vink, et al., 2001), which is in line with the findings presented in this chapter. However, other studies find no such differences between the sexes in terms of morningness-eveningness score (Caci, et al., 2009; Paine, et al., 2006). It has been suggested that, physiologically, females experience an advance in the acrophase (peak time) of the circadian rhythm of around 1 hour as compared to males (Tankova, Adan, & Buela-Casal, 1994), and it is perhaps likely that this small objective difference between the sexes is not captured by all subjective measures. However the inconsistencies in the results suggest the need to

further investigate reasons for these differences between studies in terms of the presence/absence of a true sex effect.

In addition to the observed sex differences in *absolute* morningness-eveningness score in the present sample, the results of the genetic model fitting analysis indicated the presence of differences in the *variability* of morningness-eveningness score between the sexes, such that male's scores varied to a greater extent than did female's. This finding suggests that diurnal preference may be a more variable trait for males than females. Reasons for this possible variability between males yet stability between females should be investigated in future research. It is possible that this is due to the perhaps more varied lifestyles of males, whereas the lifestyles of females may be more similar, especially for those bringing up young families where sleep-wake routines may be more constrained by school and work start time.

6.5.2 Univariate genetic analysis of diurnal preference

Around half of the variance in diurnal preference was attributable to non-additive genetic effects and half to non-shared environmental effects. This is consistent with other studies of diurnal-type, where genes (in terms of broad-sense heritability) were found to account for between 44-54% of the total variability in the phenotype, with the remaining source of variance accounted for by non-shared environmental factors (like the majority of studies reported here, shared-environmental influences were absent) (Hur, 2007; Hur, et al., 1998; Koskenvuo, et al., 2007; Vink, et al., 2001). The finding that both genes and environments are significant contributors to diurnal preference is no surprise given the abundance of research supporting a role for both endogenous factors and exogenous 'zeitgebers' (environmental time cues) in the functioning of the circadian system. It is likely that there is an interaction between these processes, such that endogenous factors are first synchronised by exogenous zeitgebers

which then go on to activate the circadian clock. The biological underpinnings of the human circadian clock are well established (see Roenneberg & Merrow, 2003, for a review). The core components of the 'clock' reside in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus, which coordinates the cellular clocks of all organs and tissues throughout the body. At the subcellular level the oscillations of the circadian clock are dependent upon the activity of 'clock genes' in a reciprocal system whereby some genes control positive elements (*BMAL1* and *CLOCK*) – which drive transcription of clock genes; whilst others control negative elements (Dunlap, 1999; Reppert & Weaver, 2001). These negative elements (including *PER1, PER2, PER3, CRY1* and *CRY2* genes) control a component of a transcriptional feedback loop in which their expression is periodically suppressed by their protein products, providing negative auto-feedback to the circadian clock (Bae, et al., 2001; Dunlap, 1999; Reppert & Weaver, 2001; Wulff, et al., 2009). Like all genes, there is much variation in the clock genes and it is these genetic variations that partially account for the inter-individual differences observed in sleep timing (Roenneberg, et al., 2007). Further discussion of this topic is reserved for Chapter 7.

With regards to the environment, it is plausible that the timing of sleep, and hence an individual's preference for sleep-wake activity, may be influenced by work, social and family commitments - which to some extent dictate their sleeping schedules. Indeed, such social demands, in particular employment and having children, have a significant modifying effect on the natural predisposition to morningness or eveningness (Adan, 1992; Leonhard & Randler, 2009; Mecacci & Zani, 1983; Monk, 2005; Paine, et al., 2006; Tankova, et al., 1994). These external pressures may thus influence the development of a preference for timing activities and sleep patterns in accordance with them. Conversely, it may be that individuals with a particular chronotype select environments that compliment their sleep-wake activity preferences.

For example, night-shift workers are characteristically 'evening' oriented (Tankova, et al., 1994). Accordingly, night-shift workers may choose work which coincides with their biological clock. It is believed that their adaptability to night work is in part due to their natural circadian phase position (Tankova, et al., 1994).

Whilst it is likely that social and work constraints play a part in influencing sleep-wake activity, it is also likely that the observed circadian rhythm phase differences between morning- and evening-types occur as a result of differential exposure to environmental factors, or zeitgebers such as exposure to light - which is considered to be a primary synchronizer of the circadian clock (Duffy & Wright, 2005). The daily variation of light and darkness is a predominant zeitgeber influencing circadian rhythmicity and preferences for sleep-wake activity. The light-dark cycle is perhaps the most important external zeitgeber as it influences the timing and daily fluctuations of all other environmental cues (such as noise and silence, heat and cold) (Roenneberg, et al., 2007; Tankova, et al., 1994). Indeed, the amount of exposure to and the intensity of light is important for the entrainment of the circadian clock (Duffy & Wright, 2005). Evening-types are exposed to less morning light and more evening light, whilst the converse is true for morning-types. This consequently results in the relatively late sleep schedules in evening-types (Goulet, Mongrain, Desrosiers, Paquet, & Dumont, 2007). Accordingly, the altered position of evening-types' circadian phase may dampen the effects of other external zeitgebers (such as light) that would usually influence 'normal' sleep timing (Monk, 1990). Thus, it is appears that a host of interacting biological, environmental, and psychosocial factors are involved in diurnal preference.

6.5.3 Associations between sleep quality and diurnal preference

The current analyses demonstrate that a preference for eveningness is associated with poor sleep quality, which is consistent with previous reports (Hogben, et al., 2007; Koskenvuo, et al., 2007; Megdal & Schernhammer, 2007; Shiihara, et al., 1998; Vardar, et al., 2008). It is possible that the association between sleep quality and diurnal preference is related to intrinsic properties of the circadian system. As briefly mentioned above, the phase position of the endogenous circadian oscillator of evening-types is delayed compared to that of morning-types (Kerkhof & Van Dongen, 1996). As a result of this phase shift the core body temperature minimum in evening-types occurs much later in the night-time period than morning-types (Baehr, et al., 2000). As such, evening-types sleep on an earlier part of their temperature cycle, and their temperature nadir occurs closer to waking compared to morning-types, i.e., the phase angle between sleep and wake-time is smaller in evening-types (Baehr, et al., 2000; Waterhouse, et al., 2001). Subjective alertness is lowest near the temperature minimum (Boivin & James, 2002; Dijk, Duffy, & Czeisler, 1992), and since evening-types awaken closer to the time of this nadir this may account for their feeling less alert upon awakening. It is also possible that the difficulty awakening of evening-types leads them to infer that they have slept poorly. Of course, it is also possible that evening-types actually sleep shorter overall, given a preference for later self-selected bedtimes in combination with earlier than desired wake-times constrained by school or occupational demands (of note, there was a small but significant association between diurnal preference and sleep duration [r = .08, p<.05], indicating that evening-types slept for a shorter duration than morning-types).

Extrapolating the association between eveningness and poor sleep quality to extreme chronotypes, it may seem plausible that in individuals with circadian rhythm sleep disorders such as DSPD (characterized by extreme eveningness), the quality of

sleep would be poor. Individuals with DSPD experience severe sleep onset insomnia and find it hard to sleep prior to around 2am-6am (American Academy of Sleep Medicine, 2005). Yet despite this, the habitual sleep period is often good after sleep onset, and such individuals experience normal sleep duration and few sleep disturbances when free to choose their sleep schedule. The discrepancy between individuals with DSPD being extremely evening oriented yet experiencing overall good sleep quality may arise because of the intrinsic phase of the circadian rhythm in DSPD. Dijk and Czeisler (1994) found that a good, consolidated 8 hour sleep was only possible when sleep was initiated 6-8 hours prior to the temperature nadir. Timing one's sleep at times incongruent to one's biological clock resulted in sleep deficits and impaired sleep quality. Yet it is suggested that the endogenous phase position of the biological clock in individuals with DSPD is delayed so that their peak time of arousal and lowest temperature phase occur extremely later than usual (Dijk & Lockley, 2002). Therefore, despite sleeping later, the timing of their sleep is congruent with their biological rhythm. Attempting sleep at times such as 2-6am in individuals with DSPD corresponds with their temperature nadir, and thus results in a good sleep providing they are able to sleep for a long duration in the morning - allowing their temperature to rise before awakening. However, truly ad lib sleep schedules are difficult to attain in the real world, and this system becomes disrupted when such individuals are not free to plan their sleep-wake schedule according to their circadian phase (due to social and work constraints or societal norms). This can be seen in Shift Work Sleep Disorder, whereby sleep quality is disrupted when individuals habitually attempt sleep during the daytime (American Academy of Sleep Medicine, 2005). This demonstrates the importance of timing sleep at times congruent with one's chronotype. Empirical research examining sleep quality in individuals with ASPD and DSPD would be beneficial to refine existing diagnostic

criteria and our understanding of sleep quality-diurnality associations in extreme chronotypes.

6.5.4 Genetic influences on the association between sleep quality and diurnal preference

The most interesting and novel finding presented in this chapter is that the association between sleep quality and diurnal preference was almost entirely explained by genetic influences, and there was substantial overlap in the genes influencing both phenotypes. This suggests that the genes associated with greater eveningness preference are also associated with increased sleep disturbance. This is informative for future research into sleep quality and diurnal preference, since it suggests that genes already known to be associated with one phenotype should be considered as possible candidates for exploration with regards to the other. Extensive research has indicated that polymorphisms of the *CLOCK* 3111 T/C gene influence eveningness and sleep timing (Katzenberg, et al., 1998), and polymorphisms of *PER1, PER2,* and *PER3* influence extreme circadian preference (Archer, et al., 2003; Carpen, et al., 2005; Carpen, et al., 2006); whereas polymorphisms of both the *CLOCK* 3111 T/C gene and the transporter region of the serotonin gene (*5HTTLPR*) have been related to sleep quality (Brummett, Krystal, Ashley-Koch, et al., 2007; Deuschle, et al., 2010; Serretti, et al., 2003). In the search for genes common to both phenotypes, it has been found that homozygosity for the 5-repeat allele in the *PER3* variable number tandem repeat polymorphism is associated with both morning preference (Archer, et al., 2003; Ellis, von Schantz, Jones, & Archer, 2009; Jones, et al., 2007) and increased sleep pressure (i.e. shorter sleep latency, more theta and alpha activity in wake and REM sleep, more slow wave activity in non-REM sleep, and more slow wave sleep (Viola, et al., 2007)), indicative of good sleep quality. Accordingly, it may be beneficial to investigate the role of these genes in

both sleep quality and diurnal preference. Yet since genetic overlap was not absolute (i.e. 52% genetic overlap between the phenotypes) it is likely that there are, to some extent, different genes influencing these phenotypes – thus, unique genes should also be explored in relation to both sleep quality and diurnal preference.

6.5.5 Environmental influences on the association between sleep quality and diurnal preference

Environmental influences accounted for only a small proportion of the association between phenotypes, and overlap between environmental influences was negligible. This finding suggests that largely unique environmental factors influence sleep quality and diurnal preference. This finding is somewhat intuitive since it is unlikely that environmental factors known to disrupt sleep, for instance living near a busy road (Kageyama, et al., 2011), experiencing family conflict (Gregory, Caspi, et al., 2006), and as found in previous chapters of this thesis, experiencing negative life events, relationship dissatisfaction, and poor general health, would have an impact on preference for sleep timing. As discussed above, environmental factors which may be more likely to influence diurnal preference may be related to work and social commitments, as well as factors related to the light-dark cycle. The lack of environmental overlap further substantiates the importance of genetic factors in explaining the association between sleep quality and diurnal preference.

6.5.6 Limitations

The main limitation specific to this study regards the age of the present sample. As a plethora of research points to the possibility that diurnal preference, as well as the influence of specific genes on diurnal preference, changes with age, it is essential for future research to investigate the association between sleep quality and diurnal

preference across the lifespan. Since evidence suggests that sleep problems increase at times of significant hormonal change, such as during puberty (Knutson, 2005) and in women, the menopause (Kravitz, et al., 2003), it is possible that the association between diurnal preference and age is in part due to associations with hormonally influenced sleep disturbance. Investigation of the specificity of the associations between sleep phenotypes and age will determine firstly whether the effects of age on diurnal preference are independent of concurrent associations with sleep quality; and secondly, whether hormonal fluctuations meditate the age-diurnality relationship.

6.5.7 Conclusion

In conclusion, this chapter determined that a preference for eveningness is associated with poor sleep quality and that this association is largely under genetic control. Furthermore, the substantial overlap in the genetic influences between phenotypes suggests that the search for candidate genes influencing sleep quality should initially focus on investigating genes for which there is already evidence of an association with diurnal preference. Likewise, the search for candidate genes influencing diurnal preference should focus on those already known to be associated with sleep quality. Worthy candidates for study in relation to both sleep quality and diurnal preference are therefore likely to be polymorphisms of *CLOCK* and those of the *PER* gene family; as well as polymorphisms of serotonin genes, in particular the transporter region of 5HT (*5HTTLPR*). Further exploration of specific genotypes influencing these phenotypes will aid in the progression to understanding the complexities of sleep and the circadian system - a topic which is addressed in the following chapter.

6.6 Acknowledgments

A manuscript based on the analyses of this chapter has been published and I thank my co-authors for their contribution.

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Chapter 7: A candidate gene study of sleep quality and diurnal preference: Associations with *5HTTLPR, PER3* VNTR and *CLOCK* 3111 and interactions with negative life events

7.1 Overview

Chapter 6 determined that there was substantial overlap in the genetic influences accounting for diurnal preference and sleep quality. Accordingly, these results tell us that in order to identify genes associated with sleep quality it may be worthwhile investigating genes already known to be associated with diurnal preference, as these are likely to also influence sleep quality, given the genetic overlap between these phenotypes. The present study investigates associations between sleep quality and diurnal preference and three functional polymorphisms: *5HTTLPR*, *PERIOD3* VNTR and *CLOCK* 3111. Furthermore, gene-environment interaction was tested in order to determine whether associations between genotypes and sleep phenotypes were moderated by negative life events. There was a significant main effect of *5HTTLPR* on sleep quality, such that the 'long-long' homozygotes conferred greater risk for sleep disturbances (β = -.34, p<.01). No evidence was found for an association between *PER3* VNTR and *CLOCK* 3111 and both sleep quality and diurnal preference; and no significant gene-environment interaction with negative life events. The main effect of the 'long' *5HTTLPR* allele contradicts previous research, suggesting that perhaps the effects of this gene are heterogeneous in different populations. Failure to replicate previous research in relation to *PER3* VNTR and *CLOCK* 3111 concurs with previous research suggesting that the effects of these genes are small and may be related to population composition.

7.2 Introduction

The search for candidate genes influencing sleep has largely focussed on the role of serotonin in sleep quality (Brummett, Krystal, Ashley-Koch, et al., 2007), and the role of clock genes in relation to circadian phenotypes, including diurnal preference (for a review see Wulff, et al., 2009). Given the evidence of substantial overlap in the genetic influences accounting for sleep quality and diurnal preference from Chapter 6, it appears logical to investigate known variants of both serotonin and clock genes in relation to both phenotypes. Accordingly, this chapter investigates the association between three polymorphisms and both sleep quality and diurnal preference: a length polymorphism in the serotonin transporter (*5HTTLPR*) (including an A/G single nucleotide polymorphism (SNP) within the transporter linked polymorphic region [LPR]); a variable number tandem repeat (VNTR) in *PER3*; and a SNP in *CLOCK* in the 3' untranslated region (UTR) at position 3111 (*CLOCK* 3111). These three genetic polymorphisms were selected because they have received considerable attention in the literature in relation to sleep phenotypes in recent years.

7.2.1 *5HTTLPR*

Serotonin (5HT) is a neurotransmitter, released by the raphe nuclei in the central and peripheral nervous systems, which modulates cognition, mood, emotion, motor function and appetite (Adrien, 2002; Cools, Roberts, & Robbins, 2008; Portas, Bjorvatn, & Ursin, 2000; Ursin, 2002), and forms a fundamental part of the body's homeostatic system, driving sleep/wake behaviour (Jouvet, 1972). Serotonergic neurons are thought to be most active during wakefulness, contribute to the build up of sleep propensity, and deactivate in the transition from wake to sleep onset (Landolt, et al., 1999). The serotonin transporter gene (*SLC6A4*, [solute carrier family 6, member 4]) controls the entire serotonergic system, maintaining the homeostasis of 5HT in the brain by

mediating the removal and recycling of serotonin after neuronal activation (Murphy, Lerner, Rudnick, & Lesch, 2004). *SLC6A4* is composed of 14 exons spanning ~31 kb (kilobases) located on chromosome 17q11.2 (Lesch, et al., 1994; Murphy, et al., 2004; Nakamura, Ueno, Sano, & Tanabe, 2000). Several polymorphisms of *SLC6A4* have previously been identified, most notably a functional polymorphism in the *SLC6A4* gene-linked polymorphic region (*5HTTLPR*), consisting of a 44bp (base pair) insertion/deletion located on the 5-flanking arm ~1 kb upstream of the 5HTT gene transcription initiation site, which either contains 14- (short, 'S') or 16- (long, 'L') repeat elements (Heils, et al., 1996; Lesch, et al., 1996). The 'short' variant of the polymorphism is associated with reduced transcriptional efficiency of the 5HTT gene, leading to decreased serotonin uptake activity and thus increased availability of serotonin at the synapse (Lesch, et al., 1996; Nakamura, et al., 2000). Recent investigations have shown that the *5HTTLPR* polymorphism itself contains an A/G SNP (rs25531), located approximately 10bp from the original insertion/deletion (Hu, et al., 2005; Wendland, Martin, Kruse, Lesch, & Murphy, 2006). The presence of a 'G' SNP on the 'long' allele reduces the expression of the gene, rendering it functionally equivalent to the 'short' allele (Wendland, et al., 2006). Thus, an L_G/L_G genotype will function as an S/S homozygote, whereas an L_A/L_G will function as an S/L heterozygote. The presence of a 'G' allele on the short allele, however, does not alter its function.

The 'short' allele has repeatedly been associated with anxiety, neuroticism and depression traits (for example, see Collier, et al., 1996; Lesch, et al., 1996), however, a substantial amount of research on this polymorphism to date has focussed on its interaction with environmental factors to influence behaviour. Gene-environment interaction (GxE) exists when genetic vulnerability to a trait increases sensitivity to environmental stressors (i.e. genetic vulnerability is modified under certain environmental conditions). With regards *5HTTLPR,* numerous studies report that the

association between the 'short' allele and psychopathology is moderated by the experience of environmental adversity. For example, the 'short' allele has been associated with greater symptoms of depression and suicidal tendencies in individuals experiencing negative life events or environmental risk (Caspi, et al., 2003; Eley, Sugden, et al., 2004). However, there is significant controversy within this field, with three recent meta-analyses providing contradictory results (see section 1.6.4.2 for further details of these studies).

Despite this surge of interest in *5HTTLPR*, few studies to date have investigated the role of *5HTTLPR* in sleep quality or diurnal preference. The only investigations of *5HTTLPR* in relation to sleep quality (to the author's knowledge) have shown that the 'short' allele is associated with primary insomnia (Deuschle, et al., 2010), and interacts with the stress of caregiving to bring about poor sleep quality (Brummett, Krystal, Ashley-Koch, et al., 2007). In relation to diurnal preference, there is evidence to suggest that serotonin activity is related to circadian rhythm regulation, and indeed one of the densest networks of serotonergic nerve endings in the brain is located in the suprachiasmatic nucleus – the area central to the regulation of circadian rhythms (Ursin, 2002). Although little, if any, research has investigated the role of serotonin specifically in diurnal preference, one study examined the differences in platelet 5HT content, 5-hydroxyindolacetic acid (5HIAA – a serotonin metabolite) content, and the distribution of *5HTTLPR* genotypes, between daytime workers and rotating shift workers (Sookoian, et al., 2007). The authors found that both platelet 5HT and 5HIAA content were significantly reduced; and that the 'short' allele of the *5HTTLPR* polymorphism was significantly more prevalent in rotating shift workers compared to daytime workers. Since night-shift workers are more typically 'evening' oriented (Tankova, et al., 1994), it may be possible to extrapolate from this latter finding to suggest that the 'short' allele of the *5HTTLPR* polymorphism may be associated with a preference for eveningness.

Given the dearth of research assessing the role of the *5HTTLPR* polymorphism in sleep quality, replication is paramount, and extending this to an investigation of diurnal preference is warranted given the lack of investigation to date. Furthermore, no studies to the authors' knowledge have investigated *5HTTLPR* and sleep with consideration of the functional A/G SNP within the LPR, and so investigation of this is essential.

7.2.2 *PER3* VNTR

The *PERIOD (PER)* gene family regulates the oscillations of the circadian clock in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Reppert & Weaver, 2001). The *PER3* gene, located on chromosome 1p36.23, is a component of the negative transcriptional feedback loop in which the gene's expression is periodically suppressed by its protein products (CLOCK and BMAL1), providing auto-feedback to the circadian clock (Bae, et al., 2001; Dunlap, 1999; Reppert & Weaver, 2001; Wulff, et al., 2009). *PER3* has been identified as a more robust marker of circadian oscillations compared to *BMAL1* and *PER2* (Archer, et al., 2008). *PER3* has two common variants of coding region VNTR in exon 18, characterised by a long allele consisting of 5-repeated 54bp of the coding sequence and a shorter allele consisting of 4-repeats (Ebisawa, et al., 2001). The 5-repeat allele has been associated with extreme morningness preference (Archer, et al., 2003; Ellis, et al., 2009; Jones, et al., 2007; Pereira, et al., 2005); decreased cognitive performance following sleep loss (Groeger, et al., 2008; Viola, et al., 2007); and increased sleep pressure - indicated by shorter sleep latency, more theta and alpha activity in wake and rapid eye movement (REM) sleep, and more slow wave activity in non-REM sleep (Viola, et al., 2007). Thus, it appears that the 5-repeat allele exerts an influence over indices of sleep intensity and quality as well as circadian timing. Conversely, the 4-repeat allele has been associated with extreme evening preference and

delayed sleep phase syndrome (DSPS) (Archer, et al., 2003). However, it should be noted that results are inconsistent and in a Brazilian population the 5-repeat allele was found to be associated with DSPS (Pereira, et al., 2005). The present study aims to replicate previous research showing an association between extreme diurnal preference and *PER3*, and investigates this in the normal range as well as in the extremes (i.e. by selecting the 10%[2] of subjects scoring the highest and lowest on the sleep measures) and to extend this to an investigation of sleep quality given the scarcity of research explicitly assessing the latter association.

7.2.3 *CLOCK* 3111 T/C

CLOCK forms part of the positive regulator of the circadian system, and acts as a transcriptional regulator that drives the expression of negative elements (*PER1, 2* and *3,* and *CRY1* and *2*) of the feedback loop (Wulff, et al., 2009). A recent study found that variants of the *CLOCK* gene, located on chromosome 4q12, are associated with sleep duration (Allebrandt, et al., 2010). Furthermore, a SNP in the 3' UTR of *CLOCK* at position 3111, consisting of a 'C' to 'T' nucleotide substitution (rs1801260), has been associated with several sleep phenotypes relevant to both sleep quality (in the context of mood disorders) and diurnal preference. Specifically, the 'C' allele is associated with shorter sleep duration and delayed sleep onset in bipolar depressed patients (Benedetti, et al., 2007); reoccurrence of insomnia in depressed patients (Serretti, et al., 2003); and a preference for eveningness in the general population in the USA (Katzenberg, et al., 1998) and Japan (Mishima, Tozawa, Satoh, Saitoh, & Mishima, 2005). However, there have also been non-replications of the latter finding (Johansson, et al., 2003; Pedrazzoli, et al., 2007; Robilliard, et al., 2002; Voinescu, Thome, & Orasan, 2009), suggesting the

[2] The study by Archer et al., (2003) selected the 7% of individuals scoring high and low on the MEQ. Here, the 10% extreme cases were selected in order to maximise power. However, 7% and 15% extremes were also tested and the results were substantially similar to those presented.

need to further investigate this polymorphism in order to gain a clearer picture of the presence/absence of an effect. No studies to the author's knowledge, however, have investigated the relationship between *CLOCK* 3111 and sleep quality in a general population sample.

7.2.4 Gene-environment interaction (GxE)

Given that recent genetic research has highlighted the importance of investigating the possibility of GxE (Moffitt, et al., 2005), GxE was also explored in the present study (in addition to examining the main effects of these genetic polymorphisms on sleep). Ignoring the possibility of GxE may lead to incorrect conclusions about the importance of genes if their effects only manifest in particular environments. In the present study self-reported negative life events was selected as a candidate environmental measure to include in the analyses, since the experience of negative life events has previously been found to moderate genetic liability to psychopathology (Caspi, et al., 2003), and is known to be associated with sleep difficulties, evidenced by the results of Chapter 5 of this thesis, as well as numerous empirical papers (Gregory, Caspi, et al., 2006; Lavie, 2001; Mezick, et al., 2009; Sadeh, 1996; Vahtera, et al., 2007). Although the results of Chapter 5 found no evidence for *statistical* gene-environment interaction between negative life events and sleep quality, investigation of *measured* gene-environment interaction is warranted as including measured genotypes increases statistical power (this is because in statistical gene-environment interaction analyses the measure of genetic influence is estimated from the pattern of cross-twin correlations on a particular phenotype, rather than objectively specifying genotype). Evidence for variability in sleep between individuals exposed to similar levels of negative life events would suggest that there may be individual variability in genetic susceptibility to sleep disturbance. Conversely, variability in sleep between individuals

with the same genotype but differing in their exposure to negative life events would suggest the presence of GxE processes.

7.2.5 Gene-gene interaction (GxG)

As well as working in an additive manner, genes are known to interact to influence behaviour – a process known as epistasis. Gene-gene interactions are known to be associated with numerous disorders (Murphy, et al., 2004). For example, Sookoian and colleagues recently reported an interaction between *5HTTLPR* and a haplotype of the *CLOCK* gene on risk for a number of symptoms related to metabolic syndrome in a sample of shift workers (Sookoian, Gianotti, Burgueno, & Pirola, 2010). Furthermore, Pedrazzoli and colleagues found that a specific combination of four CLOCK genes (*PER2, PER3, CLOCK* 3111 and *BMAL1*) was a strong marker of chronotype (Pedrazzoli, et al., 2010). Accordingly, in addition to investigating gene-environment interactions, gene-gene interactions between all permutations of the 3 genetic polymorphisms were also investigated in the present study.

7.3 Method

7.3.1 DNA collection and extraction

During previous waves of data collection (waves 1 to 3), cheek swab kits, containing 10 cotton wool buds, a 15ml tube containing a storage buffer, and a pre-paid return envelope, were posted to all study participants in order to collect DNA. DNA was provided by some but not all participants at previous waves, and so individuals participating at wave 4 who had not yet provided DNA were sent a cheek swab kit. Two postal reminders were sent in order to maximise the response rate. Cheek swabs are used to collect DNA from buccal cavity cells simply by rubbing the cotton-buds around the inside of the mouth. Cheek swabs were then placed in the tube containing storage

buffer and mailed back to the laboratory. To extract DNA from the tubes several steps were necessary as outlined by Freeman and colleagues (1997). First, the DNA was released by the activation of proteinase K by incubating the samples at around 65°C for 2 hours. Second, the tubes were centrifuged at 1000rpm to recover all of the liquid for 5 minutes. Third, using a Biomex-FX robotic-liquid handler the DNA was purified, and then centrifuged so that any debris was removed. Fourth, the DNA supernatant was manually decanted, and finally re-suspended in water by the robot. The DNA samples were then tested for degradation and purity by spectrophotometry and gel electrophoresis, and any impure or degraded samples were excluded. The genomic DNA samples were then diluted with Te buffer (10mM Tris.HCl, pH 8.0 and 1mM EDTA, pH 8.0) using the robotic-liquid handler to give a universal final volume of 100µl and final concentration of 25ng/µl, or 10ng/µl for samples that had lower original concentrations.

7.3.2 Participants

DNA from buccal swabs was collected from a total of 1,237 of the G1219 sample from all waves, of which 947 were wave 4 respondents (61% of those targeted at this wave; mean age = 20.3 years [SD=1.77], age range 18-27 years; 61.8% female). Only those participants who participated in wave 4 were included in the present analyses as this was the first wave to include information on sleep.

7.3.3 Measures

Sleep quality was assessed by the PSQI global score, as outlined in Chapter 3 (section 3.3.2). Diurnal preference was assessed by the MEQ as outlined in Chapter 6 (section 6.3.2). The PSQI and MEQ were analysed in the full range as in previous chapters of this thesis, however, in addition to using the continuous scales, the PSQI and

MEQ scores were also dichotomized to represent individuals scoring in the top and bottom 10% extremes on these scales in order to examine the extreme good and poor sleepers, and extreme morning- and evening-types in the sample, respectively (see section 7.3.5 for further details). This was done in order to directly replicate the study by Archer and colleagues (2003), which assessed the effect of *PER3* on extreme diurnal preference. In the present study, the effects of all genes on sleep quality and diurnal preference were assessed both in the full range and in the extreme. Dependent and independent negative life events were assessed by items from the 'List of Threatening Experiences' (Brugha, et al., 1985), and the 'Coddington Stressful Life Events Scale' (Coddington, 1984), as outlined in Chapter 4 (section 4.3.2.2). The number of negative life events experienced were categorised in order to investigate the influence of experiencing zero compared to any number of negative life events.

7.3.4 Genotyping

7.3.4.1 *5HTTLPR*

A multiplex polymerase chain reaction (PCR) procedure, as outlined by Wendland et al. (2006), was performed to simultaneously genotype *5HTTLPR* as well as the A/G SNP within the LPR. The PCR assays consisted of 25ng (1µl) genomic DNA, 10µl PCR master mix (2X: HotStarTaq DNA polymerase, 6nM MgCl2 buffer [pH8.7], dNTP mix [5mM of dATP, dCTP, dGTP and dTTP]: Qiagen), 6.4µl H_2O and .4µl/200nM concentration oligonucleotide primers (MWG, London; outlined in **Table 7.1**) in a final volume of 20µl per reaction. The PCR procedure was followed by double restriction endonuclease digestions using HpaII and BccI, which identifies the A/G SNP within the LPR. The alleles were categorised as 'long' (L) or 'short' (S) as outlined by (Lesch, et al., 1996). The A/G SNP within the LPR allows the distinction between S_A, L_A and L_G alleles. As the L_G allele behaves equivalently to the S allele (Hu, et al.,

2006), tri-allelic genotypes were re-categorised into a bi-allelic model according to their expression as follows: L_GL_G and SL_G were categorised as SS; SL_A and L_GL_A as SL; and L_AL_A as LL. The genotypes were visualised by gel electrophoresis as outlined in Chapter 2 (section 2.9.3).

7.3.4.2 *PER3* VNTR

A standard PCR procedure was carried out using 50ng (2 µl) genomic DNA, 10x PCR buffer IV (750mM Tris.HCl, pH 8.8; 200mM $[NH_4]_2SO_4$: ABgene), 40mM $MgCl^2$, .25µl dNTP mix (25µl/100mM concentration of dATP, dCTP, dGTP and dTTP), .2µl Taq polymerase (5 units/µl, ABI Amplitaq, Applied Biosystems), 5.7µl H^2O, and .25µl/20µM concentration oligonucleotide primers (SIGMA, Dorset; Table 7.1) in a final volume of 10µl per reaction. Alleles were categorised as 4- or 5- repeat sequences as in previous reports (Archer, et al., 2003). The genotypes were visualised by gel electrophoresis as outlined in Chapter 2 (section 2.9.3).

7.3.4.3 *CLOCK* 3111T/C

A standard PCR procedure for use with the Taqman sequence detection system (SDS) was carried out using 25ng (1 µl) genomic DNA, 2.5µl 2x ABsolute QPCR ROX Mix (ABgene, UK), .375µl H^2O, and .125µl/20µM concentration oligonucleotide primer and probe mix (ABgene, UK; see **Table 7.1**) in a final volume of 5µl per reaction. Alleles were categorised as either containing the 'T' or 'C' SNP. The genotypes were visualised using the Taqman allelic discrimination system, as outlined in Chapter 2 (section 2.9.4). Primer sequences and PCR conditions for all genes are outlined in **Table 7.1**.

7.3.5 Data preparation

In order to examine the genotypic frequencies between extreme good vs. poor sleeper groups, and the morning- vs. evening-type groups, in addition to considering scores on the PSQI and the MEQ in the full range, dichotomous variables were created prior to analysis. The frequency distributions of scores on the PSQI and MEQ were examined, and individuals scoring in the extreme top and bottom 10% of the distributions were categorised as good- vs. poor sleepers, and morning- vs. evening-types, respectively. For the PSQI, individuals scoring ≤ 2.33 were categorised as extreme good sleepers, and those scoring ≥ 10 as extreme poor sleepers. For the MEQ, individuals scoring ≤ 37.5 were categorised as extreme evening-types, and those scoring ≥ 59 were categorised as extreme morning-types. (Of note, extreme variables were also created cutting off the top and bottom 7% and 15% of the distributions. Analyses were substantially similar to those examining the 10% extremes, and so only results from the analyses examining the 10% extremes are presented). The dependent and independent negative life event variables were also dichotomised to indicate whether an individual had experienced 0 or 1+ life events. This was considered the most appropriate method of assessing life events, given that the majority of individuals had experienced either none or 1 (mean = 1.22; SD = 1.54, see **Table 5.1**).

7.3.6 Statistical Analyses

7.3.6.1 Analyses of the full range of scores

All statistical analyses were performed in STATA (Stata, 2002). First, differences in mean scores on measures of sleep quality and diurnal preference were assessed between the sexes using t-tests. Second, linear regression analyses were conducted to assess main effects of genotype on the sleep measures. Third, models were run which included main effects of genotype, negative life events (dependent and

independent, separately), and interaction terms between genotype X negative life events. Fourth, three-way and two-way gene-gene interactions on the sleep measures were tested by creating interaction terms between each combination of genes. In all regression analyses age and sex were first entered into the models, followed by main effects of genotype, main effects of dependent/independent negative life events, followed finally by the GxE and GxG interaction terms as appropriate. All analyses were run on the total sample as well as by males and females separately in order to investigate possible sex differences (in these analyses sex was not included in the regression models). Because the sample included individuals from the same families, all of the statistical analyses were conservatively corrected for the non-independence of the twin/sibling observations using the *'robust' cluster* command in STATA, as is standard in analyses of this type (see Rogers, 1993; Williams, 2000, for more information). The weight variable as described in Chapter 2 (section 2.10) was incorporated into the regression analyses in order to account for initial selection bias and attrition between waves 1-4. Of note, incorporating the weight variable resulted in substantially similar results to un-weighted analyses.

7.3.6.2 Analyses of categorical variables

Chi-squares were calculated in order to assess main effects of genotype on the frequencies of cases categorised as extreme good vs. poor sleepers and extreme morning- vs. evening-types. Additionally, chi-squares were calculated to assess whether there were main effects of each allele (rather than bi-allelic genotypes) on the good vs. poor sleeper groups and morning- vs. evening-type groups.

Table 7.1. Protocol for genotyping *5HTTLPR* (A/G), *PER3* VNTR and *CLOCK* 3111

Gene	Primer Sequences	PCR conditions (^{o} = degrees Celsius)		Product separation
5HTTLPR (A/G)	F: 5' TCCTCCGCTTTGGCGCCTCTTCC 3' R: 5' TGGGGGTTGCAGGGGAGATCCTG 3'	*Initial PCR* 95^{o} – 15 minutes 94^{o} – 30 seconds 63.4^{o} – 90 seconds } repeat 35x cycles 72^{o} – 60 seconds 72^{o} – 10 minutes	*Restriction enzyme digestion* 37^{o} – 6 hours	3.5% Agarose gel electrophoresis stained with ethidium bromide
PER3 VNTR	F: 5' CAAAATTTTATGACACTACCAGAATGGCTGAC 3' R: 5' AACCTTGTACTTCCACATCAGTGCCTGG 3'	94^{o}– 2 minutes 94^{o} – 30 seconds 69^{o} – 30 seconds } repeat 40x cycles 72^{o} – 45 seconds 72^{o} – 2 minutes		2% Agarose gel electrophoresis stained with ethidium bromide
CLOCK 3111	F: 5′-AGCCAGCAGGAGGTGATCATA-3′ R: 5′-CAGGCACCTAAAACACTGTCAGA-3′ Probe VIC-5′-ACTGGCTATGCCCC-3′ Probe FAM-5′-CTGGCTGTGCCCC-3′	50^{o} – 2 minutes 95^{o} – 15 minutes 95^{o} – 15 seconds } repeat 40x cycles 60^{o} – 60 seconds 72^{o} – 2 minutes		TaqMan ABI 7900HT Sequence Detection System; Allelic Discrimination Program (Applied Biosystems)

7.4 Results

7.4.1 Descriptives

Table 7.2 displays the genotype frequencies for the total sample (note that although 947 wave 4 participants were genotyped, the actual number of successful genotypes obtained was slightly less than 947 due to insufficient DNA. Actual numbers of successful genotypes obtained for each of the genes are indicated in **Table 7.2**). The frequencies of genotypes conformed to Hardy-Weinberg equilibrium (*5HTTLPR*: χ^2 = .07, p = .79; *5HTTLPR* (A/G): χ^2 =.80, p = .37; *PER3*: χ^2 = 2.82, p =.09; *CLOCK*: χ^2 = 2.51, p =.11).

Table 7.2. Genotype frequencies (% in parenthesis)

Marker	Genotype	Frequencies
5HTTLPR	LL	273 (32.1%)
	SL	414 (48.7%)
	SS	163 (19.2%)
	Total n	850
5HTTLPR (A/G)	LL	208 (24.5%)
	SL	438 (51.5%)
	SS	204 (24%)
	Total n	850
PER3 VNTR	4/4	395 (45.6%)
	4/5	364 (42%)
	5/5	108 (12.5%)
	Total n	867
CLOCK 3111	TT	510 (55.1%)
	TC	341 (36.9%)
	CC	74 (8%)
	Total n	925

Note. 5HTTLPR = bi-allelic genotypes based on the standard classification; *5HTTLPR* (A/G) = tri-allelic genotypes which considered the A/G SNP within the LPR, categorised into a bi-allelic model as follows: L_GL_G and SL_G genotypes were re-categorised as SS; SL_A and L_GL_A as SL; and L_AL_A as LL.

In the total genotyped sample there were significant sex differences in morningness-eveningness (mean = 47.08 [SD = 8.50]; and 49.37 [7.96], for males and females respectively, $t(940) = -4.16$, $p < .001$), indicating that females were more morning-oriented than males. There were no sex differences in sleep quality (mean = 5.59 [2.96] and 6.00 [3.17], for males and females respectively, $t(924) = -1.94$, $p = .05$). **Table 7.3** displays the mean sleep quality and diurnal preference scores by genotype for the total sample as well as for males and females separately.

7.4.2 Linear Regression Results

The linear regression analyses demonstrated that there was a significant main effect of *5HTTLPR* genotype (both the standard classification as well as when considering the A/G SNP within the LPR) on mean sleep quality score (for example, *5HTTLPR* (A/G): $\beta = -.34$, $p = .005$), indicating that LL homozygotes had significantly higher mean sleep quality scores (for example, *5HTTLPR* (A/G) mean = 6.35, SD = 3.36) (indicative of poorer sleep quality) than carriers of at least one S allele (for example, *5HTTLPR* (A/G) mean = 5.67, SD = 2.96; effect size $d = .22$) (see **Table 7.4** for a summary of the linear regression results). This result remained significant after correcting the *p*-value for multiple testing ($p = .05/8 = .006$). When assessing males and females separately the effect of *5HTTLPR* genotype (both the standard classification as well as when considering the A/G SNP within the LPR) on sleep quality was significant only in males (for example, *5HTTLPR* (A/G): $\beta = -.60$, $p = .003$, $d = .40$). There were no significant main effects of *5HTTLPR* genotype on mean diurnal preference score. Furthermore, there were no significant main effects of *PER3* or *CLOCK* on sleep quality or diurnal preference, both in the full sample and when males and females were assessed separately; and no significant interactions between all genotypes and dependent/independent negative life events on sleep quality or diurnal preference.

Table 7.3. Mean PSQI and MEQ scores (SD in parenthesis) by genotype

		Mean Score					
		Pittsburgh Sleep Quality Index			Morningness-Eveningness Questionnaire		
Marker	Genotype	Total	Males	Females	Total	Males	Females
5HTTLPR	LL	6.27 (3.28)	6.23 (3.13)	6.29 (3.36)	48.40 (8.68)	48.18 (8.50)	48.52 (8.79)
	SL	5.69 (3.07)	5.48 (2.98)	5.84 (3.12)	48.37 (8.01)	46.62 (8.27)	49.53 (7.62)
	SS	5.47 (2.61)	5.14 (2.71)	5.72 (2.52)	48.52 (8.23)	46.71 (8.78)	49.86 (7.58)
	Total n	830	323	507	847	326	521
5HTTLPR (A/G)	LL	6.35 (3.36)	6.59 (3.33)	6.23 (3.38)	48.14 (8.47)	47.34 (8.82)	48.56 (8.28)
	SL	5.76 (3.10)	5.43 (2.89)	5.98 (3.22)	48.65 (8.23)	47.46 (8.17)	49.40 (8.20)
	SS	5.47 (2.60)	5.21 (2.75)	5.67 (2.47)	48.17 (8.15)	46.13 (8.71)	49.67 (7.40)
	Total n	830	323	507	847	326	521
PER3	4/4	5.88 (3.09)	5.61 (2.94)	6.05 (3.18)	48.42 (8.13)	47.44 (8.42)	49.03 (7.89)
VNTR	4/5	5.88 (3.14)	5.68 (3.18)	6.00 (3.13)	48.75 (8.16)	47.07 (8.49)	49.69 (7.84)
	5/5	5.97 (3.12)	5.92 (2.94)	6.01 (3.27)	48.35 (9.19)	47.38 (9.08)	49.01 (9.28)
	Total n	848	322	526	864	325	539
CLOCK 3111	TT	5.86 (3.23)	5.71 (3.19)	5.96 (3.26)	48.53 (8.22)	46.96 (8.79)	49.57 (7.67)
	TC	5.86 (2.93)	5.45 (2.64)	6.09 (3.06)	48.56 (8.10)	47.14 (8.11)	49.32 (8.01)
	CC	5.88 (3.01)	5.69 (2.68)	6.02 (3.25)	47.64 (8.99)	47.30 (7.79)	47.91 (9.90)
	Total n	905	350	555	921	352	569

Note. PSQI = Pittsburgh Sleep Quality Index; MEQ = Morningness-Eveningness Questionnaire; n = total number of participants successfully genotyped that also provided complete data on PSQI/MEQ.

The results of the linear regression analyses investigating the presence of gene-gene interactions on the sleep measures are presented in **Table 7.5**. There was a significant gene-gene interaction between *5HTTLPR* and *PER3* on sleep quality, however, this result reduced to non-significance when correcting the *p*-value for multiple comparisons ($p = .05/14 = .004$). All other gene-gene interactions were non-significant.

7.4.3 Chi-square analyses of extreme PSQI and MEQ scores

The chi-square analyses of main effects of genotype on the frequencies of cases in the extreme good vs. poor sleepers, and the extreme morning- vs. evening-types are presented in **Table 7.6**. There were significant differences between the *5HTTLPR* genotypes (both the standard classification as well as when considering the A/G SNP within the LPR) and the frequencies of good vs. poor sleepers. When assessing the frequency distributions for the good vs. poor sleeper groups by allele frequency (rather than genotype), there were significantly more poor sleepers carrying at least one 'L' allele than there were good sleepers (χ^2 (1) = 9.68, $p<.001$; and χ^2 (1) = 8.94, $p<.01$ for the standard classification and A/G SNP, respectively). There were no main effects of *5HTTLPR* genotype on the extreme diurnal preference groups. Furthermore, there were no significant differences in the frequencies of cases categorised as good vs. poor sleepers or morning- vs. evening-types in genotype or allele frequency for any other genes.

Table 7.4. Standardised regression coefficients β(SE) from linear regression analyses for main effects of genotype on sleep quality and diurnal preference and interactions with dependent (dep.) and independent (ind.) negative life events

Marker		*Sleep Quality*			*Diurnal Preference*		
		Main Effects	*Genotype X dep. life events interaction*	*Genotype X ind. life events interaction*	*Main Effects*	*Genotype X dep. life events interaction*	*Genotype X ind. life events interaction*
5HTTLPR	(SS vs. SL, vs. LL)	**-.30 (0.11)****	-0.15 (0.34)	-0.64 (0.34)	-0.01 (0.34)	0.31 (1.01)	0.02 (0.97)
	(LL vs. SL, SS)	**-.34 (0.12)****	0.09 (0.24)	-0.50 (0.24)	0.04 (0.32)	0.51 (0.68)	0.13 (0.66)
	(SS vs. SL, LL)	-0.18 (.11)	-0.20 (0.17)	-0.14 (0.16)	-0.05 (0.34)	-0.14 (0.53)	-0.09 (0.45)
5HTTLPR (A/G)	(SS vs. SL, vs. LL)	**-0.32 (0.11)****	-0.21 (0.36)	-0.77 (0.36)†	-0.05 (0.34)	0.67 (1.07)	0.24 (1.04)
	(LL vs. SL, SS)	**-0.34 (0.12)****	0.04 (0.26)	-0.58 (0.26)†	0.18 (0.33)	0.40 (0.76)	0.12 (0.74)
	(SS vs. SL, LL)	-0.07 (0.10)	-0.19 (0.18)	-0.20 (0.16)	-0.26 (0.33)	0.26 (0.54)	0.11 (0.47)
PER3 VNTR	(4/4 vs. 4/5 vs. 5/5)	0.07 (0.11)	0.19 (0.30)	-0.76 (0.33)†	-0.03 (0.34)	-1.00 (0.89)	0.13 (0.96)
	(4/4 vs. 4/5, 5/5)	0.08 (0.11)	0.20 (0.21)	-0.39 (0.21)	0.06 (0.31)	-0.63 (0.55)	0.08 (0.55)
	(5/5 vs. 4/5, 4/4)	-0.03 (0.11)	0.05 (0.31)	0.63 (0.33)	0.14 (0.36)	0.75 (1.03)	-0.13 (1.05)
CLOCK 3111	(T/T vs. C/T vs. C/C)	0.00 (0.12)	-0.21 (0.29)	-0.04 (0.29)	-0.29 (0.32)	0.69 (0.82)	0.51 (0.83)
	(T/T vs. C/T, C/C)	-0.01 (0.12)	-0.16 (0.19)	-0.05 (0.18)	-0.20 (0.30)	0.45 (0.50)	0.52 (0.48)
	(C/C vs. C/T, T/T)	-0.03 (0.13)	0.04 (0.35)	-0.10 (0.36)	0.32 (0.34)	-0.40 (1.16)	0.60 (1.20)

Note. All regression analyses included the weight variable analyses to account for attrition between waves 1-4. Significance of the results were tested: ** = p <.01 after correction for multiple comparisons; † = p <.05, which reduced to non-significance when correcting for multiple comparisons.

Table 7.5. Standardised regression coefficients β(SE) from linear regression analyses of gene-gene interactions on sleep quality and diurnal preference

Marker	Sleep Quality	Diurnal Preference
5HTTLPR x *PER3* x *CLOCK*	-.26 (.30)	.76 (.82)
5HTTLPR (A/G) x *PER3* x *CLOCK*	-.20 (.31)	.87 (.86)
5HTTLPR x *PER3*	-.80 (.40) †	.47 (1.29)
5HTTLPR x *CLOCK*	.45 (.41)	.71 (1.27)
5HTTLPR (A/G) x *PER3*	-.66 (.41)	.16 (1.37)
5HTTLPR (A/G) x *CLOCK*	.54 (.43)	1.07 (1.34)
PER3 x CLOCK	-.06 (.36)	-.10 (1.15)

Note. All regression analyses included the weight variable analyses to account for attrition between waves 1-4. Significance of the results were tested: † = p <.05, which reduced to non-significance when correcting for multiple comparisons.

Table 7.6. Genotypic frequencies for the extreme sleep quality and diurnal preference groups and chi-square analyses

		Sleep Quality Groups			
Marker	*Genotype*	Good Sleepers (%)	Poor Sleepers (%)	Total (%)	χ^2
5HTTLPR	LL	18 (20.9%)	54 (47.4%)	72 (36%)	
	SL	53 (61.6%)	45 (39.5%)	98 (49%)	
	SS	15 (17.4%)	15 (13.1%)	30 (15%)	
	Total n	86	114	200	15.03 (2) p = .00
5HTTLPR	LL	11 (12.8%)	43 (37.7%)	54 (27%)	
(A/G)	SL	57 (66.3%)	53 (46.5%)	110 (55%)	
	SS	18 (20.9%)	18 (15.8%)	36 (18%)	
	Total n	86	114	200	15.49 (2) p = .00
PER3 VNTR	4/4	37 (44.6%)	57 (47.5%)	94 (46.3%)	
	4/5	35 (42.2%)	51 (42.5%)	86 (42.4%)	
	5/5	11 (13.2%)	12 (10%)	23 (11.3%)	
	Total n	83	120	203	.55 (2), p = .76
CLOCK 3111	TT	51 (57.3%)	75 (59.5%)	126 (58.6%)	
	TC	31 (34.8%)	42 (33.3%)	73 (34%)	
	CC	7 (7.9%)	9 (7.1%)	16 (7.4%)	
	Total n	89	126	215	.12 (2) p = .94

Table 7.6 (continued). Genotypic frequencies for the extreme sleep quality and diurnal preference groups and chi-square analyses

		Morningness-Eveningness Groups			
Marker	*Genotype*	Morning Types (%)	Evening Types (%)	Total (%)	χ^2
5HTTLPR	LL	36 (35.6%)	29 (29.6%)	65 (32.7%)	
	SL	44 (43.6%)	50 (51%)	94 (47.2%)	
	SS	21 (20.8%)	19 (19.4%)	40 (20.1%)	
	Total n	101	98	199	1.19 (2) p = .56
5HTTLPR	LL	23 (22.8%)	22 (22.4%)	45 (22.6%)	
(A/G)	SL	55 (54.4%)	51 (52%)	106 (53.3%)	
	SS	23 (22.8%)	25 (25.6%)	48 (24.1%)	
	Total n	101	98	199	.21 (2) p = .90
PER3 VNTR	4/4	48 (44.9%)	41 (42.3%)	89 (43.6%)	
	4/5	44 (41.1%)	41 (42.3%)	85 (41.7%)	
	5/5	15 (14%)	15 (15.4%)	30 (14.7%)	
	Total n	107	97	204	.17(2), p = .92
CLOCK 3111	TT	67 (59.8%)	57 (53.8%)	124 (56.9%)	
	TC	34 (30.4%)	36 (33.9%)	70 (32.1%)	
	CC	11 (9.8%)	13 (12.3%)	24 (11%)	
	Total n	112	106	218	.87 (2) p = .65

7.5 Discussion

The aim of the present study was to investigate associations between *5HTTLPR*, *PER3* VNTR and *CLOCK* 3111 polymorphisms and both sleep quality and diurnal preference. There was evidence for a main effect of the *5HTTLPR* 'long' allele on poor sleep quality in males. There was no evidence however for associations between *5HTTLPR* and diurnal preference. Additionally there were no associations between *PER3* VNTR or *CLOCK* 3111 and sleep quality or diurnal preference; no

interactions between any of the genotypes and negative life events on sleep; and no significant gene-gene interactions.

7.5.1 The role of the *5HTTLPR* 'long' allele and poor sleep quality

To the author's knowledge, this is the first study to report an association between the *5HTTLPR* 'long' allele and poor sleep quality in the general population. Of relevance to this, however, one study investigating the role of *5HTTLPR* in relation to the modulating effects of anti-depressant medication on motor response activity, demonstrated that 'long-long' homozygotes displayed significantly increased night-time motor activity compared to carriers of at least one 'short' allele (Putzhammer, et al., 2005). Although the study by Putzhammer and colleagues (2005) did not assess sleep quality, increased night-time motor activity is often observed in poor sleepers (Lemke, Puhl, & Broderick, 1999), and so it may be plausible to interpret Putzhammer and colleagues' (2005) finding as demonstrating a possible role for the LL genotype in poor sleep.

However, the finding that the 'long' allele confers greater risk for sleep problems is contrary to two recent studies explicitly investigating associations between *5HTTLPR* and sleep. One study found that the 'short' allele was associated with poor sleep quality in chronic stress (Brummett, Krystal, Ashley-Koch, et al., 2007); and furthermore the 'short' allele was significantly more prevalent in individuals with primary insomnia than controls in another study (Deuschle, et al., 2010). The present finding may reflect the possibility that the effect of *5HTTLPR* in the general population differs to individuals under chronic stress or with clinically diagnosed primary insomnia. Thus, the 'short' allele may only confer greater risk for sleep disturbance in extreme populations. Within the field of psychiatry, despite the more commonly held view that the 'short' allele confers greater risk for problems (for

example, Caspi, et al., 2003), a number of studies report an association with the 'long' allele and difficulties such as anxiety, depression and alcohol misuse (Baune, et al., 2008; Chipman, et al., 2007; Chorbov, et al., 2007; Gillespie, Whitfield, Williams, Heath, & Martin, 2005; Laucht, et al., 2009). Thus, as in mood disorders, it is possible that the effects of *5HTTLPR* on sleep is heterogeneous. Furthermore, since the finding in the present sample only remained significant in males when assessing males and females separately, it is possible that the effect of *5HTTLPR* on sleep is sex-dependent.

Additionally, we cannot ignore the possibility that interaction with stress may underlie associations with the 'short' allele and sleep quality. Although there was no evidence for an interaction between *5HTTLPR* and negative life events, it may be that only concurrent stress rather than distal life events moderate the association between *5HTTLPR* and sleep quality. Indeed, studies have demonstrated that the effect of stressful life events on mood (including depression) shows a temporal relationship – being strongest around one month after a stressful event occurring, and diminishing with time (Uher & McGuffin, 2008). However, this temporal relationship appears to fluctuate depending on the particular stressful event in question. Given the dearth of research investigating environmental moderation of *5HTTLPR* on sleep quality, further investigation of gene-environment interaction, considering the possibility of a temporal relationship, is essential in this context.

7.5.2 No association between *PER3* VNTR and sleep

With regard to *PER3* there was no evidence for an association with sleep quality, and the present study did not replicate a previously reported finding that the 5-repeat allele is more frequent in extreme morning-types. This is somewhat surprising

given the numerous replications of the association between the 5-repeat allele and morning preference reported in the literature (Archer, et al., 2003; Ellis, et al., 2009; Jones, et al., 2007; Pereira, et al., 2005). Previous findings such as those reported by Archer and colleagues (2003) assessed the effect of *PER3* in selected extreme morning and evening types. In the present study, there was no evidence of an effect of *PER3* in the extremes or in the full range. The extremes analysis in the present study had a larger sample size than that of Archer et al., (2003), with ~68% power to replicate the effect. Although the sample size in the present study may appear to be satisfactory to detect a small effect (e.g. $\Phi = .18$, in the paper by Archer et al., 2003), it is possible that failure to replicate the effect in the extremes was simply due to a lack of power given that power was less than 100%. In studies with small effect sizes non-replications are inevitable (Gorroochurn, Hodge, Heiman, Durner, & Greenberg, 2007). In line with our findings, however, preliminary results from a small Romanian sample reported no effect of *PER3* VNTR on sleep quality or diurnal preference (Voinescu, Coogan, & Thome, 2010). These results highlight the need for further investigation of factors influencing the association between diurnal preference and *PER3* in large samples both in the full range as well as in extreme morning/evening types.

7.5.3 No association between *CLOCK* 3111 and sleep

With regards to *CLOCK* 3111, there was no evidence for main effects of genotype on sleep quality or diurnal preference. The literature on *CLOCK* 3111 and sleep quality to date has focussed on clinical samples of depressed and bipolar patients (Benedetti, et al., 2007; Serretti, et al., 2003) and suggests that the 'C' allele is associated with greater sleep difficulties. The lack of association found here suggests that the effects of *CLOCK* 3111 on sleep quality may be specific to mood disorders

and not present in the general population. For diurnal preference, one study suggests that the 'C' allele is associated with a tendency towards eveningness and the 'T' allele with morningness in a general population sample (Katzenberg, et al., 1998). Although the larger sample used in the present study meant that there was sufficient power to replicate this effect (~80% to detect an effect size of d = .30, as reported by Katzenberg et al 1998), the present results do not support the hypothesis that *CLOCK* 3111 is associated with diurnal preference. In accordance with these findings other studies also found no evidence for this association (Johansson, et al., 2003; Pedrazzoli, et al., 2007; Robilliard, et al., 2002; Serretti, et al., 2010; Voinescu, et al., 2009). Despite this we should not rule out the possibility that *CLOCK* 3111 does contribute to the phenotypes under study, but should highlight that its effects may be too small to identify within the present sample. Furthermore, recent evidence points to the possibility that the association between *CLOCK* 3111 and diurnal preference may be dependent on its interaction with other clock genes (Pedrazzoli, et al., 2010).

7.5.4 Alternative explanations for the present results and study limitations

There are several alternative possible explanations for the present contradictory/null results which concern features specific to the study. First, a large proportion of the sample comprised university students (41.9%). It is possible that at university there is a tendency to stay up and get up late regardless of one's biological chronotype due to the lack of a fixed routine. Thus, biological effects may be masked by environmental social pressures in these participants. However, the analyses were also run after excluding university students and, while the sample size became particularly small, results mirrored those presented.

Second, the participants were younger than those participating in previous studies assessing *5HTTLPR* and sleep quality (mean age of 20 years in the present

sample; 45.7 years for cases and 54.6 years controls in the study reported by Deuschle et al (2010); and 60.9 years for caregivers and 55.8 years for controls in the study reported by Brummett et al (2007a)); those investigating *PER3* and diurnal preference (mean age of 35, 40, 24 and 25 years reported by Archer et al., 2003; Ellis et al., 2009; Jones et al., 2007; and Pereira et al., 2005, respectively); and those investigating *CLOCK* 3111 (mean age of 45 and 47 years reported by Benedetti et al., 2007, and Serretti et al., 2003, respectively). Indeed, sleep quality and diurnal preference are known to change with age (Carrier, Monk, Reynolds, Buysse, & Kupfer, 1999; Carskadon, et al., 1982; Kramer, et al., 1999), as is the effect of *PER3* on diurnal preference (Jones, et al., 2007). Thus, differences between studies in the age of participants could explain the mixed results, and it is possible that the effects of these genetic polymorphisms on sleep are age-dependent. (Of note, as age was entered into each of the regression models, the effect of age on the present results was statistically accounted for).

Third, a related point is that if the associations between genes and sleep are dependent on a gene-environment interaction effect with negative life events (most plausibly in the case of sleep quality), it is possible that the relatively young participants in the present study had not experienced the necessary and sufficient number of events for the interaction to emerge. Indeed, of the participants included in the present sample around half had experienced no dependent or independent negative life events (with the average being around 1, see Chapter 5, Table 5.1). Furthermore, the present analyses only considered the possibility of gene-environment interaction by assessing the contribution of negative life events to the associations between the genotypes and sleep. A wider scope of environmental measures should be investigated to fully determine the presence/absence of gene-environment interaction for the genotypes and phenotypes under study.

Fourth, it is possible that being a twin masks the associations between these genes and sleep. For example, it is conceivable that twins discordant in their diurnal preferences alter their sleep patterns in accordance with their co-twin. Thus, an evening-type twin whose co-twin prefers to go to bed early may disrupt the sleeping schedule of their co-twin, to the extent that the co-twin alters their diurnal preference (although it is noteworthy that the means and variances of twins compared to non-twin siblings in the sample were not significantly different, and the non-independence of the data in the analyses was statistically controlled, so the use of twins here should not be cause for concern).

Fifth, an additional consideration is that the majority of participants completed the questionnaire between March and September. It is possible that our participants had a greater preference for eveningness compared to other studies given the longer daylight hours at this time of year. Indeed, it has been suggested that seasonal variations are associated with changes in the neuroendocrine system, which may influence circadian rhythmicity (Wehr, 1998). Although there were no significant differences in mean sleep quality or diurnal preference score for those completing the questionnaire in different months, our sample's overall mean diurnal preference score was slightly lower (indicating a greater overall trend towards eveningness) than those reported in other studies (Archer, et al., 2003; Jones, et al., 2007). Although these explanations are plausible, it is possible that our contradictory findings are the result of a random sampling effect, and thus a lack of power. This highlights the necessity of replication in larger samples before firm conclusions can be drawn. Furthermore, specific characteristics of this study may account for the observed results and should be explored in further studies, and consideration of the limitations of the analyses in this thesis in general is important to this end.

7.6 Acknowledgements

A manuscript based on the analyses of this chapter has been published and I thank my co-authors for their contribution.

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Chapter 8: General Discussion

8.1 Overview

This chapter begins by summarising the results of this thesis. The limitations which are relevant to numerous chapters within this thesis are then outlined. A discussion of how the research findings of this thesis contribute to the current literature and our understanding of the aetiology of the sleep quality is then presented. Finally, possible avenues for areas of further research are offered followed by the overall conclusions of this thesis.

8.2 Summary of Results

The overall aim of this thesis was to exploit the twin design to provide a detailed account of the aetiology of sleep quality in young adults. Chapters 3-6 have taken advantage of the twin design in different ways (for example, using univariate and multivariate genetic models; liability threshold models; MZ differences analyses; models of rGE and GxE) in order to fully utilise the rich and detailed dataset. Chapter 7 has taken these analyses a step further by examining the effects of measured genes on sleep quality and diurnal preference.

8.2.1 Genetic and environmental influences on global sleep quality and the overlap in the individual components of sleep quality

Chapter 3 provided a basis for the remainder of the PhD by assessing the extent to which genetic and environmental influences contribute to the variation in global sleep quality. Furthermore, it assessed the relative contribution of these sources of variance and their overlap in the underlying liability of the severity of disturbances

in individual components of sleep quality. The aim was to determine whether the underlying contribution of genetic influences would support the use of the global score or whether, genetically speaking, the individual components measure distinct constructs in their own right.

The relative proportions of genetic and environmental influences on global sleep quality, as well as the majority of the individual components, was in line with previous studies – with genetic influences accounting for around half of the observed variability in the phenotypes with the remainder due to the non-shared environment. However, this was not true for sleep duration - where there was no evidence for genetics. This was a somewhat surprising finding, but one that can be explained in large part due to sample specific characteristics (such as the age and possible lifestyle of the participants). Evidence of strong correlations between components suggested that these components do indeed measure an underlying construct of 'sleep quality'. The substantial genetic correlations between components provided further support that the components, stemming from similar biological mechanisms, measure aspects of the same trait – justifying the use of the global score for the remainder of the analyses in this thesis.

8.2.2 Specific non-shared environmental influences on sleep quality

Chapter 4 sought to further understand the role of the non-shared environment on sleep quality, given that the previous chapter identified the non-shared environment as a significant source of influence. This study was the first to use the MZ twin differences approach to investigate *specific* non-shared environmental influences on sleep. Because of the known phenomenon that genetic influences can to some extent contribute to what we would usually consider to be 'environmental' experiences (a process known as gene-environment correlation), the MZ twin differences design

allows us to tease apart the effects of genes and environments to reveal effects that are purely environmental in origin (that is, free from gene-environment correlation). By examining the associations between a range of 'environmental' influences and sleep quality, this chapter determined that only relationship satisfaction in females, and general health in males had a purely non-shared environmental effect on sleep – that is, that the effects of all other variables (most importantly dependent negative life events) were intertwined with genetic effects. This latter point is perhaps the most important in the context of this thesis as this study has provided a novel way of indentifying gene-environment correlation.

8.2.3 Dependent negative life events and sleep quality: an investigation of gene-environment interplay

Given that Chapter 4 indentified a possible gene-environment correlation effect on dependent negative life events, and that associations between dependent negative life events and sleep quality were perhaps the strongest of all the 'environmental' variables investigated, Chapter 5 aimed to formally test the presence of rGE. In addition, Chapter 5 investigated the possibility of GxE given that an abundance of research over the past decade has found significant interactions between negative life events, genetic susceptibility and psychopathology. This was the first study to exploit the twin method in such a way as to investigate GxE in the presence of rGE in the context of sleep. The results confirmed those of Chapter 4, providing evidence for rGE between dependent negative life events and sleep quality – a finding which suggests that there may be similarities in the genetic influences affecting both sleep and the possibility of experiencing a negative life event. Mechanisms through which this may occur include the possibility that poor sleep interferes with later executive functioning, leading one to influence their own negative experiences

through limited cognitive resources. Further investigation of the consequent effects of poor sleep on the probability of experiencing negative life events is warranted in order to support this hypothesis. Of course, the converse is also likely to be true – that genetic susceptibility to experiencing negative life events consequently disrupts sleep. There was no evidence, however, for GxE. Despite this, it is possible that GxE exists in the context of sleep, but that a wider range of 'environmental' stressors should be investigated in order to observe this effect.

8.2.4 Associations between sleep quality and diurnal preference

The previous two chapters focussed on unravelling the effects of the environment on sleep quality. Chapters 6 and 7, however, turned the focus to genetics. Before delving into the vast amount of DNA and aimlessly hunting for associations between sleep and polymorphic regions, one way to begin the search for genetic variants responsible for the variation in sleep quality is to assess other phenotypes that we already have knowledge of to provide clues as to where we should focus our investigation. Accordingly, Chapter 6 assessed the association between sleep quality and diurnal preference – another sleep phenotype which shows considerable variation between individuals in the general population which is known to have significant associations with sleep quality. Like previous research, poor sleep quality was associated with a greater preference for eveningness. Most importantly, the association between sleep quality and diurnal preference was largely accounted for by genetic influences, and there was substantial genetic overlap between these phenotypes. This finding is particularly important as it suggests that the genes influencing sleep quality are shared with those influencing diurnal preference. Accordingly, these results suggest that it may be worthwhile to investigate

polymorphisms known to be associated with diurnal preference in relation to sleep quality (and vice versa) – providing us with a basis upon which to begin our search for the genetic determinants of poor sleep.

8.2.5 Associations between *5HTTLPR, PER3* and *CLOCK* 3111 and sleep quality and diurnal preference

Chapter 7 drew upon the results of Chapter 6 to investigate associations between genetic polymorphisms known to be associated with diurnal preference (*PER3* and *CLOCK* 3111) in relation to sleep quality; and to further provide support for the role of *5HTTLPR* in relation to both phenotypes given that this polymorphism has received considerable attention with regards sleep over recent years. Furthermore, this chapter aimed to determine whether the genetic effects on sleep were moderated by the experience of dependent negative life events – a test of *measured* GxE. The only significant effect between any gene and phenotype was between *5HTTLPR* and sleep quality – homozygosity for the longer allele conferring greater risk for sleep disturbance. Although this finding is contrary to much of the published literature, it is possible that sample specific characteristics accounted for the observed direction of effects. Accordingly, replication in larger and more varied samples is warranted before conclusions as to the effects of *5HTTLPR* on sleep can confidently be drawn.

8.3 Limitations

Limitations which are specific to individual chapters are addressed in the appropriate chapters. Limitations which are relevant to the overall thesis more generally are discussed here.

8.3.1 Twin Studies

Perhaps one of the most important considerations of this thesis centres on the fact that the sample used consisted of a population of twins (see Chapter 2, section 2.3 for an outline of the assumptions of the twin method). Although twin studies such as G1219 provide a perfect opportunity to examine the relative contribution of genetic and environmental influences, twin studies have been criticised on the grounds that twins may be unrepresentative of non-twins, and so extrapolation of the results of this thesis to the general population may be questioned (see Plomin, et al., 2008). Indeed, there are several ways in which this may be of concern for the present study. For example, it is possible that twins share a bedroom, and so the sleep of one twin may be disturbed by their co-twin. In terms of the timing of sleep, it is possible that twins sharing a bedroom go to bed and get up at similar times to their co-twin. Thus, a true indication of their preferences for sleep-wake activity may be masked by the fact that their sleep schedules are partly influenced by the sleeping schedules of their co-twin. However, it is likely that the twins in the present study, being between the ages of 18 and 27, were not in fact sharing a bedroom with their co-twin. Although around half of all twins included in the study were living at home with their parents as well as their co-twin, it is possible that they did not share a bedroom (unfortunately data on bedroom sharing was not available). However, it is particularly noteworthy that one study investigating the comparability between twins' and non-twins' psychiatric symptoms found that such individuals did not appear to differ on measures of insomnia and other psychiatric symptoms (Kendler, et al., 1995). Furthermore, in all analyses (both quantitative genetic analyses as well as the molecular genetic analyses) the non-independence of the twin data (i.e. twins and siblings were from the same family) was statistically controlled, mitigating this limitation as a cause for concern.

A related point regarding the use of twins is that of sibling interaction. Sibling interaction describes a process of social interaction whereby one twin's behaviour influences that of their co-twin. These effects can take two forms: cooperative (in which twins imitate each other's behaviour) and competitive (in which twins actively behave differently to their co-twin) (Boomsma, 2005). In the twin design, the presence of cooperative sibling interaction would artificially inflate the genetic correlations between MZ and DZ twins such that correlations are similar for both types of twins. Such a pattern would usually be indicative of shared environmental effects, and so it is possible that this effect will be misinterpreted as the shared environment rather than as a sibling interaction. In the context of the present study, although it is possible that the sleeping habits of one twin influenced the sleeping habits of the co-twin (thus indicating the presence of a cooperative sibling interaction effect), this should not be of cause for concern here as the general patterns of twin correlations between MZ and DZ twins did not indicate the presence of shared environment, and so this could not have been incorrectly defined in the place of a sibling interaction effect. Competitive sibling interaction, on the other hand, would create a larger discrepancy in the genetic correlations between MZ and DZ twins – leading to a pattern of twin correlations typically indicative of genetic non-additivity (also known as dominance). The presence of sibling interaction effects can be assessed by examining the variance/ co-variance structures for MZ/DZ twins. If these are somewhat similar between the two types of twins, the presence of a sibling interaction effect is unlikely. This was the case for the results of the present study, and so a sibling interaction effect was not incorporated in the formal genetic model fitting analyses. Furthermore, the power to distinguish between competitive sibling interaction and genetic non-additivity requires sample sizes much greater than that of the present sample (Reitveld, Posthuma, Dolan, & Boomsma, 2003). However, this point is worthy of consideration for future studies

assessing sleep as the presence of such effects can lead to the incorrect interpretation of the patterns of twin correlations – and hence the relative importance of genetic and environmental influences.

A final consideration of the present sample concerns the inclusion of non-twin siblings in the analyses. As twins are exactly the same age whereas siblings inevitably differ in age, it could be argued that siblings experience more divergent environments than twins. As such treating them similarly to DZ twins (for example, the equations inputted into the structural equation models were statistically identical for DZ twins and siblings, and so the calculation of their respective parameter estimates was identical) may be inappropriate. However, although it could be argued that treating DZ twins and siblings identically may be problematic, it is worth noting that the sibling correlations were generally of similar magnitude to the DZ twin correlations in most analyses. Accordingly, it can be concluded that siblings and DZ twins in the present study were not qualitatively different for the phenotypes under investigation. Furthermore, in all genetic model fitting analyses, data were regressed for age and sex and so any age effects were partialled out prior to modelling.

8.3.2 Sample characteristics

8.3.2.1 Age

The participants in the present study were between the ages of 18 and 27 years, clustering around 20 years, which limits extrapolation of the current findings to young adults only. Sleep is known to change with age (see Chapter 1, section 1.4.1; Chapter 6, section 6.5.1; and Chapter 7, section 7.5.4 for further details) and so the findings reported here may only be applicable to the age group under study. Although it is well established that sleep changes with age, it is also likely that the factors accounting for the observed variability in sleep changes with age. For example, in the present sample

there was no evidence for a role of the shared environment in sleep quality, whereas it has previously been found that in children aged between 3-4 years old, the shared environment accounted for around 70% of the variance in sleep disturbance (Gregory, Eley, O'Connor, & Plomin, 2004). However, as stated in Chapter 3 (section 3.5.4) as the age of the participants in the present sample was homogenous, it was not possible to investigate the change in heritability as a function of age.

It is also likely that age moderates the aetiological influences on diurnal preference. Indeed, preliminary reports have found that the contribution of the non-shared environment on diurnal preference decreases as a function of age in a sample of adult twins aged between 18 and 93 years from the University of Washington Twin Registry (Barclay, Watson, Golberg, & Buchwald, in preparation). It is suggested that as one ages, the decrease in importance of the non-shared environment is driven by the decline in social, work and family responsibilities as one ages, allowing the endogenous circadian rhythm to re-emerge. Further to this, it has also been shown that the effects of specific genetic polymorphisms on diurnal preference are age-dependent, being stronger in specific age cohorts as compared to others (Jones, et al., 2007). It is likely that the effects of other genes show a similar effect, the strength of the associations varying across the lifespan. This highlights the importance of confining the findings to the population under investigation.

8.3.2.2 Sex

Typically, women experience poorer sleep quality than men, as indicated by the increased prevalence of insomnia symptoms in women as compared to men in numerous populations worldwide (for example, see Ohayon, 2002). Indeed, a meta-analysis compiled data from over 30 studies of insomnia and concluded that females exhibit a predisposition for sleep disturbance (Zhang & Wing, 2006). Contrary to

much of the previous literature, however, there were no sex differences in sleep quality in the present study. As mentioned in Chapter 3 (section 3.5.1) whilst this finding was unexpected, this result conforms with other reports which have not found evidence for statistically significant sex differences in global sleep quality score measured by the PSQI (Carpenter & Andrykowski, 1998; Driscoll, et al., 2008; Valentine, et al., 2009; Valladares, et al., 2008). It is possible that the observed sex difference in insomnia symptoms is less prominent in subjectively defined sleep quality. Further investigation of the presence/absence of a true sex effect on sleep quality is required before firm conclusions can be drawn.

8.3.2.3 Ethnicity

The vast majority of the present sample considered their ethnicity as White British which limits extrapolation of the research findings to populations of other ethnic origin. This point is particularly worthy of consideration since Pereira and colleagues (2005) investigated the association between *PER3* VNTR and diurnal preference in a Brazilian population and found that the frequency of the 5-repeat allele was significantly greater in DSPS patients compared to controls - a finding which is in direct contrast to the finding by Archer and colleagues (2003) for whom the 4-repeat allele was significantly more prevalent in DSPS patients. It is suggested that one possible explanation for these contradictory findings is the ethnic background of the samples under investigation - the Brazilian population of Pereira et al. (2005) comprising individuals of European/ Portuguese/ Indian/ African/ and Asian ethnic backgrounds, as compared to the predominantly European population of Archer et al.'s (2003) study. An alternative explanation is that the latitude, and consequently the differences in day length and climate, of the two study populations explained the discrepant results. It is possible that these latitude differences affect circadian rhythm

entrainment and sleep-wake behaviour. Since the participants in the present study were recruited from a UK based twin registry, the present findings remain limited to individuals in the Northern hemisphere, with a predominantly White British ethnic background.

8.3.2.4 Attrition

In large longitudinal datasets such as G1219 attrition is unavoidable. Participants were contacted on several occasions during each wave of data collection to ensure that data was obtained from as many participants as possible, however, by Wave 4 the sample had decreased by over 50% of the original G1219Twins sample. In order to control for the effects of sample attrition from Wave 1 through to Wave 4 a response weight variable was created and used in all analyses (see Chapter 2, section 2.10 for details on the weight used in analyses). It is worth noting that all analyses were re-run without the weight variable and results substantially mirrored those presented in this thesis, and so should not be cause for concern with regards the interpretation of the findings of this thesis.

8.3.2.5 Non-clinical sample

Although the participants used in this study were not drawn from a clinical population, the focus of this study was to understand more about the factors which account for the observed variation in 'normal' sleep patterns in the general population, rather than with regard to sleep 'problems'. It is possible, however, that symptoms of sleep disorders, such as insomnia, lie on a quantitative dimension within the normal population, with individuals suffering from more chronic and pervasive problems being at the upper extreme of this continuum. Accordingly, it may be that processes which are important in determining normal sleep patterns may be applicable to clinical

sleep disorders. Thus, insomnia may be considered the extreme of poor sleep quality, and DSPS/ASPS the extremes of diurnal preference. Indeed, epidemiological studies have demonstrated that around one third of the adult population frequently experience at least one insomnia symptom making the case that these symptoms are prevalent in the general population (Ohayon, 2002). Of course it should also be acknowledged that although the sample were not from a selected 'clinical' group, the mean PSQI score was greater than the suggested clinical cut-off for a clinically relevant sleep problem (score >5) as suggested by Buysse and colleagues (1989). It seems unlikely that the present sample had a high prevalence of sleep disorders, and so the present results suggest the possibility that the clinical cut-off should be refined.

8.3.3 Self-report measures

A fundamental limitation of the present study is the reliance on self-report measures to determine sleep quality and diurnal preference. One particular consideration is that subjective reports may be sensitive to cognitive and perceptual biases. As suggested by Gerhman and colleagues (2011) 'bad' nights of sleep may be particularly salient and consequently lead an individual to retrospectively judge their sleep as poor regardless of the fact that they may also have experienced a number of 'good' nights sleep during the same period. Additionally, Gerhman notes that subjective measures of sleep may suffer from the inherent problem of asking participants to report on a state of "...reduced consciousness and awareness", and furthermore, subjective reports may be influenced by other factors such as current mood state. These biases may challenge the reliability of such measures. Of course, objective measures of sleep would be useful additions in order to better characterise the phenotypes under study, yet, the large-scale nature of the study meant that obtaining objective measures of sleep such as actigraphy or polysomnography was not

possible. Indeed others have reported that objective measures such as EEG are not suitable for large-scale studies (Liu, et al., 2000; Ohayon, et al., 2000). However, the PSQI and MEQ are among the best available methods for assessing subjectively defined sleep. They are widely used measures and good psychometric properties (including internal reliability and validity) of both are well established (Anderson, Petros, Beckwith, Mitchell, & Fritz, 1991; Backhaus, et al., 2002; Buysse, et al., 1989; Chelminski, et al., 1997; Smith, et al., 1989).

8.4 Contribution to the literature on sleep quality

8.4.1 Behavioural genetic work on sleep quality

The research in this thesis capitalises on the twin design and uses novel approaches to investigate the factors contributing to the variability in sleep quality in the general population. Since the first twin study investigating the heritability of sleep quality in 1983 (Partinen, et al., 1983), research aimed at understanding more about sleep has flourished. To date there are over a hundred papers investigating the heritability of sleep phenotypes worldwide. However, the present study is the only one to the author's knowledge to answer a *range* of questions regarding the aetiology of sleep quality using the twin design. Furthermore, it is the only study (to the author's knowledge) specifically focussed on the critical period between late adolescence and early adulthood. Accordingly, this study has replicated findings from other study populations (such as those focussing on adulthood across the lifespan) providing further support for the notion that genetic and non-shared environmental influences account for why some people sleep well whilst others sleep poorly.

8.4.2 Environmental and sociological determinants of sleep quality

In addition to contributing to the behavioural genetic literature on sleep quality, this thesis adds to the growing body of literature on the environmental and sociological determinants of sleep quality. Numerous studies have investigated societal risk factors for sleep disturbance, including low socioeconomic status, ethnicity, income, occupational status, educational attainment, and marital status to name a few (Grandner, et al., 2010; Ohayon, 2002; Patel, Grandner, Xie, Branas, & Gooneratne, 2010). In the present study, however, environmental risk factors were examined that had not been previously explored explicitly in relation to sleep quality (such as friendships, relationship satisfaction, the distinction between dependent and independent negative life events). Furthermore, the finding of significant rGE effects on the environmental variables associated with sleep provides an insight into the mechanisms by which the environment affects sleep. Accordingly, future studies should aim to unravel the determinants of the associations between sleep and the environment as the majority of the results from this thesis point to the fact that genes and environments, whilst important individually, also work in concert to bring about behaviour.

8.4.3 Molecular genetic determinants of sleep quality

Additionally, the results from this thesis add to the body of literature on the molecular genetic determinants of sleep quality. Although molecular genetic studies are increasing in number, few studies to date have investigated the role of *5HTTLPR, PER3* VNTR and *CLOCK* 3111 on sleep quality, and those that have required replication. The present research did not confirm previous findings, however this in itself is an important finding and brings up numerous questions as to why previous results were not supported in the present research. The null findings in relation to

PER3 and *CLOCK* 3111 suggest that these effects may perhaps be limited to specific populations (for example in mood disorders), may be chance effects, may be too small to replicate even in samples as large as G1219, or may be dependent on interactions with other genetic polymorphisms.

In the case of *5HTTLPR*, the contradictory finding presented here suggests that the association between this polymorphism and sleep quality is not as clear-cut as it may first seem. It is possible that the direction of its effect is dependent on the population under investigation (for example, confined to clinical sleep disorders, or to individuals experiencing chronic stress) or that it interacts with other, as yet unidentified, environmental stressors. Thus, it appears that the effects of *5HTTLPR* on sleep are diverse and require thorough investigation. Furthermore, it appears that a re-consideration of the role of serotonin in sleep is warranted, as the current theory – that since increased serotonin function is associated with wakefulness (for example, see Ursin, 2002, for a review), sleep is disrupted by *higher* levels of serotonin at critical brain regions - needs to be revisited in light of the present findings. This is because the longer allele, which results in greater re-uptake of serotonin at the receptor sites and consequently *decreases* levels of serotonin relative to the effects of the short allele, constituted a risk factor for poor sleep – contrary to the current known mechanism of action. It would be particularly useful to confirm whether long-long homozygotes in the present study do indeed have altered levels of brain serotonin during sleep compared to short allele carriers, to confirm whether the allelic effects are due to differential modulation of absolute amounts of serotonin activity.

However, it appears that understanding of the effects of serotonin on sleep is complicated for two reasons: First, it is possible that the effects of serotonin on sleep are in part dependent on its interaction with other brain neurotransmitters (such as noradrenaline and acetylcholine), since the onset of sleep is reliant on the complex

interaction of many inhibitory and excitatory processes (Portas, et al., 2000). Second, it is likely that serotonin has differential effects on sleep dependent on its localization within the serotonergic system. Indeed administration of selective serotonin re-uptake inhibitors (SSRI's) in cats has been found to have both sleep promoting and sleep incompatible effects and it is suggested that the observed effects may be due to regional differences in reuptake (Sommerfelt & Ursin, 1991). With this in mind investigation simply of the overall effect of serotonin on sleep may not be appropriate, but rather a finer examination of the role of increased/decreased serotonin in specific brain regions throughout the sleep-wake cycle may shed greater light on this complex process.

8.5 Implications of the current research

The implications of the research stemming from this thesis are threefold, impacting on scientific theory, clinical practice and the development of future research. In terms of scientific theory, the results of this thesis have increased our understanding of the factors accounting for variation in sleep-wake behaviour in healthy young adults. Furthermore, this thesis has substantiated how we conceptualise 'sleep quality'. The finding that the genetic influences on the individual components of sleep to a large extent overlapped, suggests that we can consider these different aspects of sleep as stemming from similar biological mechanisms. Not only this, but the results of this thesis have suggested that, in a similar manner, the determinants of sleep quality and diurnal preference are largely the same genetically, but vastly distinct, environmentally. The unexpected finding regarding the importance of the 'long' allele of *5HTTLPR* as a risk factor for sleep disturbance suggests the possibility of an alternative mechanism of action between serotonin and sleep to the well

established links between the 'short' allele and sleep disturbance, which should be explored further.

The finding that although sleep quality is substantially influenced by genetic factors, environmental influences are just as important is likely to be informative for the development of treatment and intervention programmes for sleep disturbances. Informing individuals of the benefits of controlling their environment (such as by maintaining good sleep hygiene, reducing exposure to high risk environments), is likely to have positive effects on their sleep. In addition, educating individuals about their own biological rhythms, understanding when their optimum times are for sleep-wake behaviour and informing them of the risks of sleeping at times incongruent with their internal biological clock, may have positive effects on their sleep and lifestyle. Furthermore, controlling lifestyle factors (such as work and social commitments) will also aid in the maintenance of a regular internal clock which is likely to have positive consequences for sleep, behaviour and psychological functioning.

The present findings may also be informative for clinicians, especially those refining diagnostic criteria for sleep-wake disorders in the next edition of the DSM in 2013. Several changes are proposed to the diagnostic criteria for Insomnia Disorder (previously termed 'Primary Insomnia'), with the inclusion of the criteria that "...the predominant complaint is dissatisfaction with sleep quantity or quality..." (American Psychiatric Association, 2010), whereas in the previous edition, the DSM described the predominant complaint as "...difficulty initiating or maintaining sleep, or non-restorative sleep, for at least one month" (American Psychiatric Association, 1994). The research presented in this thesis, focussing on understanding the aetiology of variation in subjective sleep quality, therefore provides information of relevance to the predominant clinical feature of insomnia disorder as diagnosed by DSM-V criteria.

8.6 Directions for future research

Despite answering numerous questions related to the understanding of the aetiology of sleep quality, the present research has yielded just as many new questions. The points below detail possible avenues to explore with the aim to understanding more about the aetiology of sleep. These are just some of the questions that this research has generated – it is likely that there are many more that will emerge as research into this area continues.

8.6.1 Extrapolation to other study populations

Focussing on a homogeneous age group is a strength of the present study allowing us to understand more about sleep in young adulthood. However, it is likely that a different pattern of results would emerge in different age groups. Accordingly, future research should aim at replicating the current findings across the lifespan: from infancy to childhood; childhood to adolescence; adolescence to adulthood; young to mid-adulthood; and mid-to late adulthood. Each of these milestones is associated with significant changes biologically, cognitively and socially, making it unequivocal that behaviour, and thus sleep, will change alongside them. It appears that the heritability of sleep is likely to change with age, but there are additional questions to be answered. For example, are there specific environmental influences that have a greater effect on sleep at certain points in life? Are gene-environment correlation effects sustained across the lifespan or do genetic effects on 'environmental' variables change with age? Are there significant 3-way Gene X Environment X Age interaction effects – whereby a gene-environment interaction effect is only exhibited in particular age groups?

Just as with age, where possible, future research should aim to replicate the current findings in clinical samples. This will allow us to determine whether the effects found here in relation to sleep quality and diurnal preference can be extrapolated to insomnia and circadian rhythm sleep disorders such as ASPS and DSPS. This will enable us to determine whether sleep quality is a quantitative dimension which has insomnia at its extreme. For example, it will be useful to investigate whether the same biological, physiological, sociological and cognitive determinants are important in both the normal range of sleep disturbances as well as in clinical cases, but are exacerbated in the extreme (i.e. insomnia).

Not only should the present results be examined in populations of individuals with clinical sleep disorders, but they should also be investigated in individuals with psychopathology. Since clinical problems such as anxiety and depression are so often comorbid with sleep disturbances (for example, see Morin & Ware, 1996), research should examine whether the genetic and environmental effects on sleep are the same in such individuals as compared to individuals with isolated sleep disturbances. This will be important in understanding whether comorbid sleep disturbances which are often seen as a symptom of the psychopathology rather than a diagnosis in their own right, stem from the same factors as those observed in the general population, or whether such sleep disturbances are qualitatively distinct, aetiologically.

However, it should be noted that there are numerous difficulties associated with recruiting clinical participants, the main one being that the possibility of obtaining a large twin sample comprising clinical participants is highly unlikely. Accordingly, designing alternative experimental designs to the twin design will be necessary in order to address these questions in clinical samples.

8.6.2 Stability, change, and direction of effects

Large samples of twins studied at multiple time points will afford us the opportunity to examine the stability of sleep overtime, as well as longitudinal associations between sleep and the environment, allowing us to ask questions regarding the direction of effects. For example, do sleep disturbances persist from young adulthood to mid-adulthood? If so, what accounts for this stability? Such data will allow us to determine whether the genetic and environmental effects on sleep are stable across time, or whether there are new factors which come into play at different time points. In relation to associations between sleep quality and diurnal preference, does having an evening-type personality, or factors associated with an evening-type personality (such as consuming alcohol, interacting with friends late at night) lead one to sleep poorly, or does the fact that an individual sleeps poorly mean that they delay going to bed? Furthermore, is this effect genetically or environmentally mediated? In relation to associations between sleep and the environment, do negative life events lead to poor sleep, or is the converse true? It is likely that both explanations are true, but what determines this? Answering such questions may be important for theoretical models of sleep disturbance, allowing us to understand whether the experience of poor sleep itself is instrumental in influencing our behaviour and environmental experiences.

8.6.3 Molecular genetic methods

Given the likelihood that many genes of small effect are responsible for sleep-wake behaviour it is no surprise that the search for genes influencing sleep has been slow. Replication of the present results, both the negative and positive findings, is essential in order to further substantiate the claims made here.

8.6.3.1 Serotonin

The somewhat surprising finding that the longer allele of the *5HTTLPR* gene was associated with poorer sleep quality brings up numerous questions worthy of clarification. First, is this a robust finding? Since this is the only study to date investigating the role of *5HTTLPR* in relation to sleep quality in a sample of healthy individuals, this finding should be considered as preliminary. Further studies are required before we can begin to answer this question. Second, if this finding is robust, how can it be explained? Seeing as the consequences of this finding being true is somewhat at odds with the current literature on the role of serotonin in sleep and wakefulness, further *in vivo* experiments explicitly focussed on the effects of increased/decreased serotonin in primary brain centres for sleep-wake activity (such as the SCN, as an example) would provide useful insights into the plausibility of this finding. Third, is it possible that other *5HT* related polymorphisms exhibit a similar effect – i.e. leading to decreases in brain serotonin in individuals experiencing sleep disturbances? Such an investigation would highlight whether this effect is specific to the transporter gene, or whether it is true of genes across the entire serotonergic system more generally.

8.6.3.2 Other genes

In order to find clues as to where to search for other candidate genes contributing to sleep quality, future research should use the approach of Chapter 6 to look at associations between sleep quality and other phenotypes. For example, research from G1219 has identified significant genetic correlations between sleep quality and externalising behaviours (such as aggressive behaviours and rule breaking behaviours: Barclay, Eley, Maughan, et al., 2010). Thus, we should perhaps focus our

search for genes implicated in sleep quality on those known to be associated with externalising behaviours. One possible candidate for further investigation is the monoamine oxidase-A (MAO-A) gene. Low levels of brain MAO-A activity has been associated with higher levels of self-reported aggression symptoms in healthy adult males (Alia-Klein, et al., 2008). MAO-A degrades the monoamine neurotransmitters serotonin, norepinephrine and dopamine. Accordingly, low levels of brain MAO-A result in excess levels of these neurotransmitters (Alia-Klein, et al., 2008). Thus, it can be seen how this may have an effect on sleep quality, given the possibility that increased levels of serotonin may lead to sleep disturbance due to its role in wakefulness (see Jouvet, 1999, for a discussion of this hypothesis). Indeed a polymorphism of the MAO-A gene, which results in less transcriptional efficiency of the gene and consequently *increased* levels of these monoamine neurotransmitters, has been associated with poor sleep quality in a small sample of males (the large majority of whom were caring for a relative with dementia) (Brummett, Krystal, Siegler, et al., 2007). Replication of this finding in much larger, more typical study populations is required before we can draw firm conclusions as to the effects of MAO-A on sleep. If this finding is robust, however, it posits the possibility that polymorphisms of norepinephrine and dopamine genes should also be targets for study in relation to sleep, given the effects of MAO-A on levels of these neurotransmitters. This is just one example of how behavioural genetic research can further refine our search for genes associated with sleep quality. Considerably more research assessing the relationship between sleep and numerous behavioural traits is likely in the coming years given the increasing number of, and wealth of data included in twin studies across the globe (including large twin studies in the UK, Sweden, Finland, and the USA to name but a few). This is likely to suggest avenues for further research not previously explored.

An additional molecular genetic method which is likely to yield positive results in mapping genotype to phenotype is genome-wide association studies (GWAS). To date there are only a handful of GWAS focussed on sleep phenotypes. One such study identified a couple of circadian clock genes as potential mediators of bedtime and sleepiness (*NPSR1* and *PDE4D*) (Gottlieb, O'Connor, & Wilk, 2007). A recent genome-wide scan of genes associated with insomnia has yielded evidence for a role of genes previously associated with bipolar disorder and schizophrenia (Ban, Kim, Seo, Kang, & Choi, 2011). Owing to their increasing availability and ever decreasing costs it is possible that we will see more and more of these studies in the coming years. Such studies are likely to lead to the exponential growth of knowledge of the genes involved in sleep. This progress is further facilitated by the better ways of accurately characterising sleep (such as PSG and actigraphy).

8.6.3.3 Endophenotypes

An alternative way to maximise the chances of successfully identifying genetic polymorphisms associated with poor sleep is to study endophenotypes. An endophenotype can be described as a "...measurable component unseen by the unaided eye along the pathway between disease and distal genotype..." (Gottesman & Gould, 2003, pg 636). Accordingly, an endophenotype may be a particular characteristic which is consistently reported to be evident in the phenotype/disorder under study, but which is not typically observed as an overt symptom of the phenotype/disorder. To qualify as a potential endophenotype, a quantitative trait must satisfy 4 criteria: first, it must be heritable; second, it must reliably be associated with the phenotype/disorder under investigation; third, it must be state-dependent (that is, it must manifest regardless of whether the phenotype/disorder under investigation is present); and fourth, it must co-segregate with the phenotype/disorder under investigation

(Gottesman & Gould, 2003). Certain characteristics of REM sleep, such as an increase of REM density and a decrease of REM sleep latency, have been suggested to be endophenotypes of depression (Gottesmann & Gottesman, 2007). Yet what may be considered endophenotypes of sleep disturbances? A recent report from The Netherlands has found disturbed intracortical excitability during waking in insomnia patients using Transcranial Magnetic Stimulation (TMS) compared to controls. Surprisingly, this excitability did not recover after treatment of the insomnia, showing that such activation was a stable trait regardless of the exhibition of insomnia symptoms, suggesting that this pattern of brain activation may be an endophenotype (van der Werf, et al., 2010). Accordingly this study demonstrates that this trait satisfies at least two of the criteria for classification as an endophenotype (association with insomnia; state-dependency). The authors suggest that genotyping the endophenotype may lead to fruitful insights into possible genetic variants associated with the broader insomnia phenotype given that this particular pattern of excitability appears to be a highly heritable trait (Pellicciari, et al., 2009). Accordingly, further research should aim at indentifying other possible endophenotypes of sleep as well as sleep disorders such as insomnia, with the hope that doing so will facilitate further progress in identifying genes responsible for such disorders.

8.6.4 The environment

The present study has focussed on a few very specific environmental measures in association with sleep, largely due to the availability of the data within the G1219 study. It is likely that there are many more to be explored given the almost infinite number of situations that we as individuals are exposed to in our everyday lives. Significant gene-environment interaction effects may emerge in studies examining a broader scope of environmental measures than encompassed here. For example, in the

study by Brummett and colleagues (2007a), the effect of *5HTTLPR* on sleep quality was moderated by the experience of caring for a relative with dementia. Indeed there are an abundance of environmental influences which may affect sleep, and the present study has touched upon just a few.

Future studies should also aim to provide more objective methods of measuring the environment. It is possible that self-reports of the environment are affected by one's subjective interpretation of their *perception* of the environment rather than the objective state of that environment (Kendler & Baker, 2007). This in itself could be considered as a completely distinct way of conceptualising the environment (i.e. the *perception* of the *experience* rather than the *exposure*). For example, an individual may interpret a particular life event as negative (e.g. moving house) which may be seen positively by others. Thus, by measuring environmental variables by more objective means (such as direct observation) may shed light onto whether the measurement of the environment is confounded by the interpretation that the participant imposes upon it.

Furthermore, much of the research on sleep and indeed on other phenotypes has focussed on environmental 'risk factors' for problems, but what about the possibility that there are environmental measures that promote positive outcomes? Do positive life events facilitate sleep? For example, does marriage serve as a protective factor against sleep difficulties? It is likely that happily married couples lead more stable, structured lives, and adhere to good sleep hygiene practices compared to single individuals who may have more of a tendency to go out with friends at night. Indeed, in adults lifestyle regularity has been found to be a protective factor for good sleep (Monk, Reynolds, et al., 2003), so it can be seen here how such an effect may occur. It is rare in epidemiological research to identify correlates of positive behaviours, but

perhaps such investigations will prove to be just as insightful as those aiming to identify factors associated with problems.

8.6.5 Conclusions

The G1219 study has provided the unprecedented opportunity to use quantitative and molecular genetic techniques to progress knowledge regarding the aetiology of sleep quality in young adulthood. Through so doing the results of this thesis have underscored the importance of genetics, but just as importantly, the environment, and the known reality that these influences work in concert to influence sleep. Although this research has addressed the specificity of these influences on sleep, future research should draw upon these techniques to further advance the knowledgebase regarding the specific genetic and environmental factors involved. It may be considered that the era of quantitative genetics is coming to an end, with the emergence of high-throughput molecular genetic techniques, and the ever decreasing costs of full genome sequencing. However, the results of this thesis affirm that the work of quantitative geneticists is not likely to disappear any time soon, given that twin studies can be used in numerous ways to answer complex questions regarding nature and nurture, and the fascinating ways in which these effects intertwine to influence every aspect of our being. Further exploration of the ways in which behavioural genetic methods can be exploited will aid in the progression to understanding the complexities of sleep and the circadian system. The future of sleep research is exciting, and new discoveries are being made everyday – even as we sleep.

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