EFFECTIVE ASSESSMENT OF ACCIDENT RISK IN

OBSTRUCTIVE SLEEP APNOEA

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This thesis contains published work and/or work prepared for publication, which has been co-authored. Outlined below are the bibliographic details of the work, estimated percentage (%) contribution of authors and where it appears in the thesis.

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Contribution by the candidate: The candidate proposed and designed the research; collected, cleaned, coded, and analysed the data; interpreted the results, and wrote the manuscript (70%). The co-authors assisted with data collection, provided input with the design and concept, guidance for statistical analysis, data interpretation and critical review of the manuscript (30%).

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Contribution by the candidate: The candidate proposed and designed the research; collected, cleaned, coded, and analysed the data; interpreted the results,
and wrote the manuscript (70%). The co-authors assisted with data collection, provided input with the design and concept, guidance for statistical analysis, data interpretation and critical review of the manuscript (30%).

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**Contribution by the candidate:** The candidate proposed and designed the research; collected, cleaned, coded, and analysed the data; interpreted the results, and wrote the manuscript (70%). The co-authors assisted with data collection, provided input with the design and concept, guidance for statistical analysis, data interpretation and critical review of the manuscript (30%).

**Declaration for thesis containing published work and/or work prepared for publication**

I confirm that permission has been obtained from all co-authors to include the manuscripts in this thesis.

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ABSTRACT

Background and aims: Obstructive sleep apnoea (OSA) is a common and under-recognized breathing disorder of sleep associated with excessive sleepiness and impaired cognition. These symptoms of sleepiness and inattention predispose individuals with OSA to increased risk of motor vehicle crashes (MVCs) and occupational accidents. However the factors that predict increased risk remain unclear, since the relationships between OSA and neurobehavioural impairment are complex and vary between individual patients.

Two studies were undertaken in this thesis to address aetiological aspects of the relationship between OSA, excessive sleepiness and accident risk. The third study evaluated a simple portable sleep monitor with the goal of expediting OSA diagnosis in individuals at high risk of accidents. The aims of this research were three-fold: (1) to describe the incidence rate of MVCs in patients with OSA, and characterise factors that predict increased risk; (2) to investigate whether genetic variants known to regulate circadian rhythm and sleep homeostasis are independently associated with excessive sleepiness in patients with OSA; and (3) to validate a simple portable sleep monitor to ‘rule-in’ OSA.

Methods: To address aims 1 and 2, this thesis utilised data from a prospective case-series of patients with OSA (n = 2,673) drawn from the Western Australian Sleep Health Study (WASHS). Recruited patients were referred to a tertiary hospital-based sleep clinic (West Australian Sleep Disorders Research Institute [WASDRI]) for assessment of a suspected sleep related disorder. A sub-set of these individuals (n = 1,301 OSA cases) had genetic data available. To address aim 3, validation of a home sleep portable monitor (PM) was conducted on
consecutive clinic patients (n = 104) with possible OSA who completed a home PM study, a PM study simultaneous with laboratory polysomnography (PSG), and a second home PM study. Primary outcomes of interest were the positive likelihood ratio (LR+) and sensitivity of the PM device to “rule-in” OSA, defined as an apnoea-hypopnoea index (AHI) ≥ 5 events per hour on PSG.

**Results:** Our study found that sleep clinic patients with untreated OSA reported MVCs at a rate three times higher than the general community (mean crash rate ratio 3.07, 95% CI 2.98 to 3.17, p < 0.0001). We investigated risk factors for MVCs and near-misses in sleep clinic patients and found a strong association between sleepiness and increased rate of reported near-misses. Very sleepy men reported near-misses 4.68 (95% CI 3.07-7.14) times more often than normal men. For women there was a significant association with sleepiness score (p = 0.02), but no dose effect across sleepiness quartiles. Genetic association analyses between single-nucleotide polymorphisms (SNPs) in the Period3 (PER3) gene and excessive sleepiness found three PER3 SNPs were associated with increased risk of dozing in a low somnificity (alerting situation, all p ≤ 0.001), after adjusting for severity of OSA and a SNP*severity interaction. These associations remained significant after multiple testing adjustment. Interaction plots for SNP rs697693 illustrate that risk of dozing was genotype-dependent and varied according to severity of OSA.

Evaluation of a two-channel portable sleep monitor found that the device has adequate LR+ (4.8), sensitivity (80%) and specificity (83%) for detecting OSA in the unattended home setting when benchmarked against laboratory PSG. There were no significant night-night (all p > 0.10) or study order effects (home or laboratory first, p = 0.08) on AHI measures. Manual PM data review improved case
finding accuracy, although this was not statistically significant (all $p > 0.07$). Misclassification was more frequent where OSA was mild.

**Conclusions:** Western Australian drivers with untreated OSA have a significantly higher risk of MVC compared with the general community. Investigation of MVC risk factors in OSA patients found a strong association between excessive daytime sleepiness and increased report of near-misses. Thus it is those patients with increased sleepiness regardless of OSA severity that are most at risk, supporting current Australian Fitness to Drive Guidelines. Novel associations of known circadian rhythm variants (Period3) with excessive daytime sleepiness in subjects with OSA have been described for the first time. These genetic associations were modulated by an interaction with severity of OSA suggesting that sleep disruption is a greater contributor to excessive sleepiness and involuntarily falling asleep than is hypoxia. Comprehensive validation of a simple home portable sleep monitor to ‘rule-in’ OSA in a high pre-test probability sleep clinic population can expedite screening of high-risk individuals. Evidence from this study lends support to the incorporation of ambulatory management of OSA into practice alongside polysomnography.
PUBLICATIONS

Publications arising directly from this thesis

Peer-Reviewed Published Articles:


Peer-Reviewed Published Conference Abstracts:


**Publications arising from candidature, to which the candidate made a significant contribution**

**Peer-Reviewed Published Articles:**


L Simpson, DR Hillman; MN Cooper; **KL Ward**, M Hunter, S Cullen; A James; LJ Palmer; S Mukherjee; PR Eastwood. High prevalence of undiagnosed obstructive sleep apnoea in the general population and methods for screening for representative controls. *Sleep and Breathing*, 2012. 16 (4)
PREFACE AND ACKNOWLEDGEMENTS

The Western Australian Sleep Health Study (WASHS)

The studies undertaken for this thesis utilised data collected for the WASHS. This large case series of sleep clinic patients was developed to create a resource to facilitate research into the genetics and epidemiology of OSA. The candidate participated in all aspects of data collection for WASHS from 2007 to 2010. I would like to thank the sleep clinic patients for their participation in this study, and to gratefully acknowledge all the students and volunteers who have assisted with the data collection for the WASHS. Special thanks go to Sutapa Mukherjee, David Hillman and Lyle Palmer for the huge effort given to establish the WASHS. The inspiration to pursue my own research project stemmed from your enthusiasm to build this great resource.

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<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AASM</td>
<td>American Academy of Sleep Medicine</td>
</tr>
<tr>
<td>AHI</td>
<td>apnoea hypopnoea index (events per hour of sleep)</td>
</tr>
<tr>
<td>ARI</td>
<td>arousal index</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index (kg/m²)</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CPAP</td>
<td>continuous positive airway pressure</td>
</tr>
<tr>
<td>DF</td>
<td>degrees of freedom</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOT</td>
<td>Department of Transport</td>
</tr>
<tr>
<td>EEG</td>
<td>electro-encephalgraph</td>
</tr>
<tr>
<td>EOG</td>
<td>electro-oculograph</td>
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<tr>
<td>EMG</td>
<td>electro-myograph</td>
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<tr>
<td>ESS</td>
<td>Epworth sleepiness score</td>
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<tr>
<td>FDR</td>
<td>false discovery rate</td>
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<tr>
<td>GLM</td>
<td>general linear model</td>
</tr>
<tr>
<td>GWA</td>
<td>genome wide association</td>
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<tr>
<td>GWAS</td>
<td>genome wide association study</td>
</tr>
<tr>
<td>hMAF</td>
<td>Minor allele frequency reported using the International HapMap Project: data release 24/phase II November 2008; European population</td>
</tr>
<tr>
<td>HWE</td>
<td>P-value from a Fishers exact test for Hardy Weinberg Equilibrium</td>
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<tr>
<td>IQR</td>
<td>inter-quartile range</td>
</tr>
<tr>
<td>kb</td>
<td>kilobases</td>
</tr>
<tr>
<td>kg</td>
<td>kilograms</td>
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<tr>
<td>LR-</td>
<td>likelihood ratio of a negative test calculated as (1-sensitivity) divided by specificity</td>
</tr>
<tr>
<td>LR+</td>
<td>likelihood ratio of a positive test calculated as sensitivity divided by (1-specificity)</td>
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<tr>
<td>MaF</td>
<td>Minor allele frequency</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>mmHg</td>
<td>millimetres of mercury</td>
</tr>
<tr>
<td>MVC</td>
<td>motor vehicle crash</td>
</tr>
<tr>
<td>n</td>
<td>number of sample</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>OSA</td>
<td>obstructive sleep apnoea</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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<tr>
<td>OSAHS</td>
<td>obstructive sleep apnoea hypopnoea syndrome</td>
</tr>
<tr>
<td>PER2</td>
<td>period circadian clock gene 2</td>
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<tr>
<td>PER3</td>
<td>period circadian clock gene 3</td>
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<tr>
<td>PSG</td>
<td>polysomnography</td>
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<td>PM</td>
<td>portable monitor</td>
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<tr>
<td>RDI</td>
<td>respiratory disturbance index</td>
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<td>REM</td>
<td>rapid eye movement sleep</td>
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<td>RR</td>
<td>rate ratio</td>
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<tr>
<td>SaO₂</td>
<td>arterial oxygen saturation</td>
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<td>s</td>
<td>seconds</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<td>standard error</td>
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<tr>
<td>SHHS</td>
<td>Sleep Heart Health Study</td>
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<tr>
<td>SNP (s)</td>
<td>single nucleotide polymorphism (s)</td>
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<tr>
<td>SWS</td>
<td>slow wave sleep</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
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<tr>
<td>WASDRI</td>
<td>West Australian Sleep Disorders Research Institute</td>
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<tr>
<td>WASHS</td>
<td>Western Australian Sleep Health Study</td>
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<tr>
<td>WSCS</td>
<td>Wisconsin Sleep Cohort Study</td>
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<tr>
<td>χ²</td>
<td>Chi-square statistic</td>
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GLOSSARY

**Actigraph**: a non-invasive sensor worn on the wrist, leg or hip that continuously monitors gross motor activity and can be used to monitor rest and activity cycles in humans.

**Allele**: alternative form of a gene that can occur at a single locus.

**Association study**: the study of association between an outcome (e.g. allele) and a trait (e.g. having OSA) in a population.

**Blood Alcohol Concentration (BAC)**: the amount of alcohol present in the bloodstream and most commonly used as a metric of alcohol intoxication for medical and legal purposes.

**Candidate gene**: a gene which can be reasonably posited to be involved in the genesis of a phenotypic trait or disease on the grounds of biological plausibility.

**Case**: an individual in which the disease or trait of interest is present.

**Chromosome**: a linear arrangement of DNA that contains genes and other proteins.

**Cleveland Family study (CFS)**: a family-based study of sleep apnoea of 2284 individuals (46% African American) from 361 families started in 1991 and followed over a period of 16 years.

**Complex disease**: a disease involving multiple genetic and environmental factors that does not exhibit a Mendelian pattern of inheritance.

**Epworth Sleepiness Score (ESS)**: an 8 point instrument assessing the likeliness of dozing in passive and active day time situations that is used as a proxy measure of day time sleepiness.

**Framingham Heart Study (FHS)**: a longitudinal study of several large cohorts of men and women from Framingham, Massachusetts in the United States started in 1948.

**Gene**: a segment of DNA on a chromosome that is inherited and contains the information required to produce a functional product via transcription to RNA and translation to amino acids.

**Genotype**: the specific combination of alleles at one locus.

**Hardy-Weinberg equilibrium**: the principle which describes the distribution of genotypes at a locus in terms of its allele frequencies in a population.
**Heritability**: the proportion of total phenotypic variation between individuals in a given population due to genetic differences. This number can range from 0 (no genetic contribution) to 1 (all differences on a trait reflect genetic variation).

**Heterozygote**: an individual having two different alleles at a given locus.

**Homozygote**: an individual having two identical alleles at a given locus.

**Hypercapnia**: where the threshold of carbon dioxide in the blood is too high. Hypercapnia is an arousal stimulus in sleep.

**Linkage disequilibrium**: the co-occurrence of alleles at two or more loci at a frequency that is greater than expected by chance.

**Locus**: the specific place on a chromosome at which a gene is located (multiple: loci).

**Multiple Sleep Latency Test (MSLT)**: a sleep disorder diagnostic tool giving an objective measure of sleep propensity. Sleepiness is conceptualised as the tendency to fall asleep by measuring the speed of falling asleep.

**Maintenance of Wakefulness Test (MWT)**: an objective test designed to measure the ability to remain awake in a somnolent situation, often used to assess safe driving ability.

**Negative predictive value (NPV)**: the proportion of subjects with a negative test who are truly negative for the condition and it is calculated as: true negatives / (true negatives + false negatives).

**Odds ratio (OR)**: the ratio of odds of a dichotomous outcome (e.g. having moderate-severe OSA) among exposed individuals relative to unexposed individuals.

**Partial sleep deprivation (PSD)**: also known as sleep restriction, refers to a reduction in the total sleep time relative to one’s usual baseline during a 24-hour period.

**Phenotype**: the set of observable characteristics (or behaviours) of an individual resulting from the interaction of their genotype with the environment.

**Psychomotor Vigilance Task (PVT)**: a sustained-attention, reaction-timed task that measures the speed with which subjects respond to a visual stimulus.

**Positive predictive value (PPV)**: the proportion of subjects with a positive test who are truly positive for the condition and it is calculated as: true positives / (true positives + false positives).
**Single nucleotide polymorphism (SNP):** a variation at a specific location in the DNA sequence due to a single nucleotide difference between individuals.

**Sleep Heart Health Study (SHHS):** a prospective cohort study of adults aged 40 years and older who have been assessed by polysomnography for sleep disordered breathing. Participants have been drawn from several parent cohort studies including the FHS.

**Total sleep deprivation (TSD):** refers to the avoidance of sleep for a period of at least one night.

**Trait:** A distinguishing characteristic typically belonging to an individual that may be genetically determined.

**Western Australian Sleep Health Study (WASHS):** an epidemiologic, genetic, and biospecimen resource created to enable research into sleep disorders, which recruited approximately 90% of all patients presenting at the Sir Charles Gairdner Hospital sleep clinic, Western Australia’s largest facility for the diagnosis and treatment of OSA and other sleep disorders.

**Wisconsin Sleep Cohort Study (WSCS):** a prospective, longitudinal study of a population-based cohort of adults aged 30 to 60 years designed to investigate the natural history of sleep disordered breathing, established in 1988.
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1 INTRODUCTION

1.1 CONTEXT OF THESIS

As with people in many other parts of the world, Australians are confronted with an epidemic of motor vehicle crashes and work related accidents.\textsuperscript{1} Much of this accident risk is attributable to sleepiness and impaired vigilance. Obstructive sleep apnoea (OSA), an important and under recognized clinical condition\textsuperscript{2} is a major cause of these symptoms\textsuperscript{3} and of impaired cognitive function.\textsuperscript{4} Indeed, OSA sufferers are at increased risk of motor vehicle\textsuperscript{5} or occupational accidents.\textsuperscript{6} Drivers with OSA are two to seven times more likely to have a motor vehicle crash than those without sleep apnoea.\textsuperscript{5} Obesity is known to exacerbate OSA and is present in approximately 70\% of subjects with OSA.\textsuperscript{7,8} It contributes to the rising prevalence over the past two decades of OSA\textsuperscript{9} and associated diseases such as diabetes mellitus.\textsuperscript{10} OSA is the most common of the primary sleep disorders which collectively were estimated to directly and indirectly cost the Australian economy $36.4 billion per annum in 2010.\textsuperscript{1}

In 1993, Young \textit{et al.} reported a substantial prevalence of symptomatic OSA in the community (2\% of women, 4\% of men),\textsuperscript{11} which was particularly high in middle-aged individuals. In the Busselton, Western Australia community approximately 10\% of middle-aged men had significant OSA.\textsuperscript{12} In 2013, an update of the prevalence of OSA for the Wisconsin cohort in the USA reported expected increases in parallel with the ongoing obesity epidemic. The revised estimates for those with OSA (apnoea hypopnoea index [AHI] ≥ 5 events/h) plus symptoms of
daytime sleepiness have more than doubled (5% of women, 14% of men). It is concerning that a comprehensive review of the prevalence of OSA has reported at least 80% of all moderate to severe OSA in men and women in the USA remained undiagnosed.

Many occupational groups, such as professional drivers, are vulnerable to OSA due to the sedentary nature of their work and its association with poor eating and sleeping habits. Consequently, obesity and OSA are particular problems for this demographic. In a large study of Australian commercial vehicle drivers, 60% of drivers had sleep disordered breathing and 24% were excessively sleepy. Crashes involving trucks and buses are particularly expensive and calamitous, and they are associated with higher fatality rates.

From a clinical perspective there is a need to understand which individuals with ‘probable OSA’ are at increased risk of motor vehicle crashes. A recent systematic review of this topic concluded that subjects with OSA are clearly at increased risk, but the characteristics that may predict crash risk in drivers with OSA are less clear. They include body mass index, AHI, hypoxia, and possibly excessive daytime sleepiness. Current fitness-to-drive guidelines include advice not to drive for persons who are very sleepy, defined as an Epworth Sleepiness Score (ESS) of ≥ 16. Although, as a group, drivers with OSA are at increased risk of crash, the risk in less sleepy individuals with OSA is not clear. In addition to effects on health and well-being, the economic burden of morbidity and mortality associated with motor vehicle crashes is high both in Australia and internationally. Therefore, it is important to clarify the risk factors associated with OSA that predict the risk of crash. This would facilitate identification of those at particular risk.
risk so that targeted preventative measures can be deployed. Recent reports on
drowsy driving in both the US and Europe have called for increased attention to
the problem of crashes due to sleepiness.\textsuperscript{18}

There is a poor correlation between symptoms of excessive sleepiness and
severity of OSA, as judged from AHI. In a 2005 study, $< 50\%$ of cases with moderate
to severe OSA reported excessive sleepiness.\textsuperscript{19} Because the sleepiness symptoms
associated with OSA vary between individuals,\textsuperscript{20} an individual diagnosed with
severe OSA may not be sleepier than one with mild disease. This conundrum can
provide a difficult management situation for a clinician with respect to adherence
to the guidelines for fitness to drive. A recent meta-analysis has shown that the
risk of motor vehicle crashes is removed when OSA subjects are treated with
continuous positive airways pressure (CPAP),\textsuperscript{21} as CPAP is an effective therapy for
the treatment for OSA of all severities. It would be of considerable practical benefit
to determine if individuals with OSA who are not overtly symptomatic (sleepy)
have an equivalent risk of crash to individuals without OSA. This would allow
better assessment of risk for occupational drivers, machinery operators and those
involved in safety-critical tasks.\textsuperscript{22}

There is appreciable inter-individual variation in the susceptibility to
sleepiness in relation to the presence of sleep disorders or extent of sleep
restriction, although this appears to be a stable trait within individuals.\textsuperscript{23} It has
been postulated that this variability plays a role in the mismatch between
sleepiness symptoms and disease severity seen in many OSA subjects.\textsuperscript{24} What are
the components of an individual sleep trait or phenotype which might define
vulnerability or resilience to sleep loss? Sleepiness, diurnal preference and sleep
duration are recognised as heritable traits, but the genetic basis is largely unknown.\textsuperscript{25-27} There have been few studies to date specifically exploring the genetics of excessive sleepiness phenotypes in the context of specific sleep disorders such as OSA, narcolepsy and advanced sleep phase disorder.\textsuperscript{26,28}

The genetics of the circadian sleep/wake cycle have been elucidated predominantly through animal model work.\textsuperscript{29} Period genes play a role in the regulation of this cycle and how it couples to day and night.\textsuperscript{30} The genetic control of sleep homeostasis is less well understood.\textsuperscript{31} Particular period gene polymorphisms have been shown to have an association with sleep phase disorders\textsuperscript{26} and it has been postulated that certain sleep phenotypes may be more vulnerable or resilient to sleep loss.\textsuperscript{24} The sleep disruption caused by OSA results in a sleep debt, often presenting as the symptom of excessive sleepiness. Certain sleep phenotypes may be particularly vulnerable to sleep debt, such as those with a long sleep requirement and evening chronotype. In such cases, it has been hypothesized that the sleep disruption of OSA confers an additional sleep loss load on an already vulnerable system, and contributes to excessive sleepiness symptomatology.\textsuperscript{32}

In addition to factors predicting crash risk, this thesis explores the association of specific period candidate genes to ‘sleepy’ phenotypes in subjects with OSA from the Western Australian Sleep Heath Study (WASHS).\textsuperscript{33} It attempts to answer the question of whether known genetic determinants of diurnal rhythm are related to sleepiness symptoms in OSA cases. It is possible that ‘sleepiness’ genes play a role in the perception of tiredness or sleepiness, and this may influence crash risk in a sleep clinic population. If such genes could be identified
1: INTRODUCTION

and shown to confer increased risk (short-term accident risk and long-term morbidity and mortality), then there is potential to develop improved risk models for the management of OSA. Recent attention has focussed on the potential for identification of a biomarker for sleepiness to assist in this regard.\textsuperscript{34,35}

The burden of undiagnosed OSA in the community is high,\textsuperscript{9,12} with OSA prevalence increasing as a result of the obesity epidemic.\textsuperscript{36} Patients with untreated OSA are more likely to have a motor vehicle crash than those without it,\textsuperscript{16} and drowsy driving is a major cause of highway accidents and fatal crashes.\textsuperscript{37-39} The most common daytime symptom associated with OSA is excessive daytime sleepiness.\textsuperscript{40} Sleep physicians are regularly confronted with the dilemma of diagnosing and treating the causes of sleepiness in occupational drivers. Given the increased risk of crashes in individuals with OSA, there is an imperative to promptly diagnose and treat this disorder. The current gold standard investigation of PSG is under-resourced relative to the prevalence of the condition.\textsuperscript{41} There is a need for validated, cost-effective diagnostic tools to expedite identification of suspected OSA cases, especially those who are very sleepy and so at increased risk of occupational and driving accidents.\textsuperscript{42}

Validation of a simple home portable sleep monitor against ‘gold-standard’ laboratory-based sleep studies has the potential to allow triage of subjects for urgent assessment and treatment of OSA. Acceptance of portable sleep monitor technology has been slow due to insufficient evidence to support their accuracy in specific populations.\textsuperscript{43,44} A recent systematic review reported that limited-channel devices must be validated in the setting of intended use and in the population to be screened, since the predictive value of a test varies with the prevalence of the
1: INTRODUCTION

condition. Existing sleep services are unable to service the large number of individuals being referred as there are insufficient sleep specialists and laboratories to deal with the large clinical burden of OSA. Thus a further goal of this thesis was to validate a simple portable sleep monitor suitable for identification of OSA cases.

In summary, work described in this thesis has the potential for significant public health benefits through the characterisation of risk factors that identify individuals with OSA at high risk of accidents. Individuals with a high pre-test probability of OSA can be promptly diagnosed with a home portable sleep study. Once identified, cases can commence treatment and so ameliorate the risk of sleepiness-related motor vehicle crashes and occupational accidents. A better understanding of the genetic basis of sleep disorders, particularly the contribution of the circadian rhythm or ‘Period’ genes may further identify those who are particularly susceptible to the effects of sleep loss. This will contribute to improved risk models for sleepiness among sleep clinic patients, particularly vulnerable occupational groups at high risk of sleepiness-related accidents.
1: INTRODUCTION

1.2 THESIS AIMS

Sleep health is an important public health preventative issue because sleep deprivation can substantially impair human health and performance. Within sleep health considerations there are three key areas of incomplete knowledge that are addressed in this thesis:

1. Description of the incidence rate of motor vehicle crashes in patients with OSA, and characterisation of factors that predict increased risk.
2. Investigation of whether genetic variants known to regulate circadian rhythm and sleep homeostasis are independently associated with excessive sleepiness in patients with OSA.
3. Validation of a simple portable sleep monitor to ‘rule-in’ OSA.

1.3 THESIS STRUCTURE

This thesis is presented in chapters that describe a series of three interrelated scientific papers (Chapters 3, 5 and 6). The thesis opens with an introduction (Chapter 1) that outlines the context of the work. Chapter 2 presents a literature review that critically assesses current knowledge of OSA, excessive daytime sleepiness and accident risk. Chapter 3 addresses aim 1 through an investigation of motor vehicle crash risk in patients with OSA. Chapter 4 reviews the genetics of sleep, with particular focus on the genes purported to regulate the sleep-wake cycle. Chapters 5 and 6 address aims 2 and 3. The thesis concludes (Chapter 7) with a summary of the main findings and discussion to contextualise the results in relation to the general field of sleep disorders research.
2 LITERATURE REVIEW

2.1 FOREWORD

This chapter provides an overview of the literature pertaining to the increased risk of driving accidents associated with OSA, the diagnostic methodology for OSA, the neurocognitive deficits resulting from sleep deprivation and the public health implications of untreated OSA in society. A review of the genetics of sleep and vulnerability to sleepiness is presented separately in Chapter 4.

2.2 THE PURPOSE OF SLEEP

Sleep is as essential to well-being as eating and drinking, and necessary to feel refreshed and perform optimally during the day.\(^4^7\) It is so crucial that organisms have evolved tightly regulated mechanisms to promote and optimise sleep at night. Normal sleep is regulated by a two-process sleep model consisting of a circadian process linking sleep to the 24 hour day-night cycle and a homeostatic process governed by time awake which builds sleep pressure.\(^4^8\) Borbély proposed this model in 1982 and conceptually it remains valid. There is growing understanding of the essential role of sleep in maintaining the vital biological functions of recovery, energy conservation and survival.\(^4^9\) The importance of sleep to neurobehavioural function is particularly evident from findings examining the effect of total and chronic sleep deprivation.\(^4^7\) Many aspects of neurobehavioural function are impaired by extended wakefulness.\(^5^0\) Despite the knowledge that adequate sleep is crucial to good health, there is a tendency in modern life to short-change sleep requirement. Many people mistakenly believe
that sleep is readily dispensable and can be caught up, and most community surveys of sleep habits reveal a consistent pattern of less sleep on week nights than on weekends.51-53

Fuelling these pressures on sleep, remarkable societal changes have occurred since the provision of artificial light and affordable energy.54 Many work sectors now run around the clock with people working shifts outside daylight hours to accommodate 24 hour operations; this is the reality for many industries such as health services, transport, shipping, mining, food delivery and emergency services. Travel, globalisation and computerisation of monetary markets, banking and commerce have further contributed to sleep loss in society. Consequently, many people work at night (then sleep during the day) in contradiction to the body's natural synchronisation to the 24 hour day-night cycle.55 There is growing concern that social and economic pressures have taken priority over the fundamental importance of adequate quantity and quality sleep for optimal health, performance and well-being.49,56

2.3 SLEEP LOSS

As well as societal demands, a wide range of other factors contribute to chronic insufficient sleep (also referred to as sleep loss, restriction and fatigue): these are mainly volitional and a consequence of suboptimal work schedules or lifestyle choices that reduce the quantity of sleep; and, less frequently, pathological, whereby a disorder is present that disturbs sleep quality.54 Sleepiness and fatigue are common in the community, with about 18 to 24% percent of people admitting to excessive daytime sleepiness or fatigue.53,57 Insufficient sleep is the largest contributor to unrefreshing sleep, and is both
extremely common (24% report inadequate sleep)\textsuperscript{50,53} and critically relevant in our society.\textsuperscript{54} The consequence of excessive daytime sleepiness, regardless of whether it is due to insufficient quantity or quality, is an increased likelihood of performance errors or adverse incidents. In many segments of society, such as public transportation, energy plants, the health system and commercial transport, such errors may have tragic consequences.\textsuperscript{58,59} For example, the grounding of the Exxon Valdez oil tanker was a high-profile catastrophe attributed to sleepiness-related human error.\textsuperscript{54} A disaster of this scale is expensive, can cause huge environmental and human damage, and undermines public trust in the transport industry.\textsuperscript{60} To prevent such tragedies, a substantial body of work has been devoted to understanding the neurocognitive consequences of sleep deprivation\textsuperscript{61} and the relevance of this work in relation to the studies in this thesis is discussed in detail in Section 1.9. A brief summary of normal human sleep and the consequence of sleep loss follows.

\section*{2.4 Normal Human Sleep}

Three interrelated factors influence the adequacy of normal sleep: sleep duration, sleep timing, and sleep quality, and adverse outcomes for health and waking function result when any of these factors are sub-optimal relative to need.

\subsection*{2.4.1 Sleep duration}

To maintain optimum health, approximately a third of each day must be spent asleep. Sleep propensity increases with time awake and eventually alertness is impaired; thus a strong driver for the need to sleep is the duration of time awake. Basal sleep need varies across age groups but most adults require 7 to 8 hours sleep per day.\textsuperscript{62} The usual sleep requirement is a very stable trait within
individuals. Early research into the association between sleep duration and mortality found a U-shaped relationship whereby mortality risk increased in individuals with average sleep durations of less than 6 hours (short sleepers) or greater than 9 hours (long sleepers). More attention has been focussed on short sleep duration since the prevalence of short sleepers has increased over the past 30 to 40 years.

The sub-group of short sleepers (habitual sleep duration < 6 hours) includes ‘true’ natural short sleepers and ‘sleep insufficiency’ short sleepers. In natural short and long sleepers, sleep length is primarily governed by genetic determinants. By contrast, ‘sleep insufficiency’ short sleepers are sleep deprived by volitional restriction, which may be social or employment-related. Multiple studies have shown that short sleep is associated with increased disease risk, including hypertension, heart disease, diabetes, obesity and cancer, as well as impaired performance and memory, impaired immune function and increased risk of accidents. However, a recent review of secular trends in adult sleep duration failed to show a consistent decrease in self-reported sleep duration, and thus the evidence for an association between short sleep duration and adverse health outcomes is not conclusive. Nevertheless there is mounting evidence that basal sleep need must be met to maintain health and function. Sleep duration is one component of an individual sleep phenotype to which both genetic and environmental factors contribute (See Chapter 4: The Genetics of Sleep).
2.4.2 **Sleep timing and regulation**

Sleep is regulated by the interaction of two oscillatory processes: the sleep homeostat and the circadian pacemaker. The timing of sleep is influenced by the relationship between these oscillations, with the circadian clock aligning our biological rhythms with day and night. The homeostat tracks sleep debt such that the duration of wakefulness is extended, sleep propensity increases and alertness is impaired. The circadian pacemaker is a self-sustained oscillator that generates and maintains the timing of behavioural and physiological events that follow a 24-hour rhythm. The most obvious circadian rhythm in humans is the cycle of wake and sleep. The circadian clock controls physiology at numerous levels, from gene expression at the cellular level to complex behaviours such as sleep and performance. The circadian profiles of core body temperature and plasma melatonin are conventional markers of this biological clock. Sleep propensity, subjective alertness and task performance rhythms are synchronised with these biological clock markers, ensuring a relatively stable level of daytime alertness with a healthy sleep-wake cycle.

These circadian rhythms are central to our sleep-wake cycle, and attempts to sleep at inappropriate phases of the cycle (e.g. during the day for night shift workers) will usually result in shorter sleep episodes, more awakenings and sleep fragmentation. It should be noted, however, that sleep can be initiated at all circadian phases as long as sufficient homeostatic pressure for sleep is present. Thus the sleep homeostat and circadian systems are interrelated and the interactions are likely bidirectional. This core model of sleep regulation applies to all humans but there are strong inter-individual differences in both the sleep
homeostatic and circadian processes likely related to genetic variations\textsuperscript{26} (Chapter 4: The Genetics of Sleep). Thus the timing of self-selected sleep is multifactorial, defined by genetic disposition (chronotype; Chapter 4), sleep debt accumulated on work days, light exposure (circadian factors) and shift work.\textsuperscript{79}

2.4.3 Sleep quality

A normal night of sleep has characteristic structure in terms of sleep stages and their proportions against which abnormalities can be compared in clinical practice.\textsuperscript{80} Several demographic, environmental and individual factors are known to modify sleep stage distribution and influence quality of sleep.\textsuperscript{62} Age is a strong determinant of the structure of sleep, although this structure is stable over short time periods.\textsuperscript{62} Environmental factors to consider within the time frame of the sleep study are ambient temperature and drug ingestion, especially alcohol in Western society. With respect to sleep regulation, sleep propensity is significantly affected by prior sleep history. Sleep loss of one or two nights will result in a rebound pattern of sleep that favours slow wave sleep (SWS) during recovery.\textsuperscript{81} In addition, deprivation of either rapid eye movement (REM) or SWS sleep will result in a preferential rebound of that sleep stage if and when normal sleep resumes. Thus in a clinical situation all of these phenomena must be considered. Circadian rhythm, the other core component of sleep regulation, is influential since both shift work and jet lag can result in a phase shift of the sleep-wake pattern.\textsuperscript{82} Finally, pathology can have a substantial effect on sleep quality, with sleep disorders and other medical conditions impacting the structure, quality and distribution of sleep. It has been estimated that poor or inadequate sleep affects over 20% of
Australians on a near-daily basis, and half of this problem is attributable to common sleep disorders such as OSA and insomnia.\textsuperscript{53}

In summary, refreshing sleep consists of adequate duration relative to usual need, timed appropriately to day and night, and of good quality. Recently the scale of health and social consequences associated with insufficient sleep and sleep disorders has been highlighted.\textsuperscript{53} The adverse effects of sleep loss extend to wellbeing, productivity and safety, and thus represent a significant public health issue.\textsuperscript{49} Sleep disorders contribute to the burden of sleep loss in society by disrupting sleep quality. Obstructive sleep apnoea is the most common medical condition that causes excessive daytime sleepiness,\textsuperscript{83} and warrants further consideration as the prevalence of the condition has increased substantially over the past thirty years.\textsuperscript{9}

\section*{2.5 Obstructive Sleep Apnoea (OSA)}

Obstructive sleep apnoea is a breathing disorder of sleep, first described in 1965.\textsuperscript{84} Sleep is disrupted by partial (hypopnoea) or complete (apnoea) closure of the upper airway during sleep, resulting in arterial oxygen desaturation and arousals from sleep. OSA is usually quantified by the AHI, the total number of episodes of apnoea and hypopnoea per hour of sleep. A value of 5 or more events/hour is most often used to define OSA.\textsuperscript{85}
2.5.1 The definition of Obstructive Sleep Apnoea Hypopnoea Syndrome (OSAHS)

The diagnosis of OSAHS requires the presence of the symptom of excessive daytime sleepiness (A) or two or more clinical features (B) (outlined in Table 2.1 below) and an AHI > 5 (C).

Table 2.1. The American Academy of Sleep Medicine Task Force Diagnostic Criteria for OSAHS

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Excessive daytime sleepiness that is not better explained by other factors</td>
</tr>
<tr>
<td>B</td>
<td>Two or more of the following that are not better explained by other factors: choking or gasping during sleep, recurrent awakenings from sleep, unrefreshing sleep, daytime fatigue, impaired concentration</td>
</tr>
<tr>
<td>C</td>
<td>Overnight monitoring demonstrates five or more obstructed breathing events per hour during sleep. These events may include any combination of obstructive apnoeas/hypopnoeas or respiratory effort related arousals</td>
</tr>
</tbody>
</table>

OSAHS, obstructive sleep apnoea hypopnoea syndrome.

A position paper based upon the Chicago diagnostic criteria (Table 2.1) proposed clinical practice guidelines in 2001. The cases reported in this thesis were defined using these clinical criteria and the Chicago criteria for respiratory data analysis. The symptom of excessive daytime sleepiness and the clinical signs were elicited by questionnaire (ESS [Section 2.7.3.2]) and clinical interview, whereas the AHI was measured objectively by overnight PSG (Section 2.5.4). The ESS is the most commonly-used rating of sleepiness in research, and is widely used in clinical practice as a means of standardising the report of sleepiness.
The symptom of excessive daytime sleepiness is common in OSA but does not have to be present (Criterion B, Table 2.1). This symptom may present as daytime fatigue or impaired concentration, both of which may impair driving, but would not necessarily result in an elevated ESS score. In several large studies, the proportion of subjects with OSA reporting excessive daytime sleepiness ranged from 16 to 22%.\textsuperscript{9,90} There is often a mismatch between the number of breathing disturbances (AHI) and arousals and the degree of excessive daytime sleepiness. This suggests that sleepiness, in part, is independent of the number of breathing pauses and that other factors play a role.\textsuperscript{91} The symptom of excessive sleepiness is central to this thesis and is discussed in detail in Section 2.7.

2.5.2 \textbf{Symptoms and clinical risk factors of OSA}

Common indicators of OSA are snoring, witnessed apnoea, hypertension, nocturia and daytime sleepiness.\textsuperscript{92} An individual with OSA may be unaware of many of the night-time signs and the physician may be aided by bed partner report. A summary of night-time signs, awake symptoms, clinical features and risk factors associated with OSA is given in Table 2.2.
Table 2.2. A summary of night-time signs, symptoms when awake, clinical features and risk factors associated with obstructive sleep apnoea.92

<table>
<thead>
<tr>
<th>Night-time signs</th>
<th>Symptoms when awake</th>
<th>Clinical features</th>
<th>Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitual snoring</td>
<td>Daytime sleepiness</td>
<td>Obesity (BMI &gt; 30 kg/m²)</td>
<td>Male gender</td>
</tr>
<tr>
<td>Witnessed apnoea</td>
<td>Waking unrefreshed</td>
<td>Increased neck circumference</td>
<td>Middle age</td>
</tr>
<tr>
<td>Restless sleep</td>
<td>Drowsiness while driving</td>
<td>Hypertension</td>
<td>Supine posture asleep</td>
</tr>
<tr>
<td>Choking arousal</td>
<td>Morning headache</td>
<td>Crowdred oropharynx</td>
<td>Positive family history</td>
</tr>
<tr>
<td>Gastro-oesophageal reflux</td>
<td>Cognitive deficits</td>
<td>Nasal obstruction</td>
<td>Alcohol ingestion</td>
</tr>
<tr>
<td>Nocturia</td>
<td>Lack of concentration</td>
<td>Narrow mandible and maxilla</td>
<td></td>
</tr>
<tr>
<td>Insomnia with frequent awakenings</td>
<td>Changes in mood</td>
<td>High, narrow hard palate</td>
<td></td>
</tr>
</tbody>
</table>

OSA, obstructive sleep apnoea; BMI, body mass index.

Shaded symptoms relate to a facet of cognitive function that may contribute to accident risk.

The risk factors most strongly or consistently related to OSA are obesity and increased age.40,93-98 With respect to obesity, male gender increases risk further since ‘android-type’ obesity (fat deposition in the neck and abdomen) worsens OSA.99 Almost all OSA subjects snore100,101 and the most common complaint is daytime sleepiness or fatigue.100,102,103 Of particular relevance to the work described in this thesis are the signs and symptoms related to excessive sleepiness since it is this component of OSAHS that is most closely related to workplace and
motor vehicle accidents, impaired social functioning and reduced quality of life. Six of the eight daytime symptoms cited in Table 2.2 (shaded) relate to a facet of cognitive function that may contribute to accident risk. Aside from the obvious symptomatic benefit to the patient when diagnosed and treated for OSAS, it is important for the clinician to be able to identify at-risk sleepy individuals expeditiously, ideally at first presentation. Chapter 6 deals with the issue of prompt identification and diagnosis of such individuals.

2.5.3 Diagnosis of OSA

A careful clinical history is a key component of the diagnosis of OSA, but confirmation with an overnight sleep study is important as the key symptoms and signs do not specifically predict OSA. Sleep experts are generally wrong in 50% of cases when making a diagnosis on history and examination alone. Snoring is present in 70-95% of OSA patients but is also common in the general population, making it a poor predictor alone. Similarly, 30-50% of the general population report moderate to severe sleepiness so excessive daytime sleepiness is a poor discriminator. Witnessed apnoea is the third common reason for referral to a sleep clinic but is rarely accurately reported, even by a bed partner. Thus diagnosis is based upon an evaluation of symptoms and clinical features plus an objective measure of sleep disordered breathing.

2.5.4 Polysomnography

The gold standard for diagnosis of OSA is polysomnography (PSG). In Australia, subjects are most often studied overnight in a laboratory where parameters of respiration, sleep quality and leg movements are recorded digitally. The methodology for PSG has been defined by the American Academy of Sleep
Medicine (AASM) guidelines. The record is reviewed interactively by a qualified technologist to generate a report that details sleep quality and respiratory disturbances. Scoring rules have been developed for both sleep staging and respiratory event scoring.

2.5.5 The scoring metric – Apnoea Hypopnoea Index

The diagnosis of OSA is confirmed by scoring the PSG to generate the apnoea hypopnoea index (AHI). This is simply the number of apnoeas and hypopnoeas recorded per hour of sleep. Thus the AHI represents an index of sleep disruption. Severity of OSA is defined according to AHI categories as follows: ‘normal’ (AHI < 5 events/h); ‘mild OSA’ (AHI ≥ 5 and < 15 events/h); ‘moderate OSA’ (AHI ≥ 15 and < 30 events/h); and ‘severe OSA’ (AHI ≥ 30 events/h). Although these categories are clearly defined, different AHI cut-off values have at times been used to indicate presence of OSA in research and prevalence studies.

The AASM developed guidelines in 1999 to standardise the scoring of respiratory events for the purpose of clinical research, and these became widely known as the ‘Chicago’ criteria. Since the original definition of the AHI, several revisions of the scoring rules for events and the measurement technologies for signals have occurred in order to standardise methodology internationally. The guidelines were revised in 2007, since it was realised that there was significant variability in the clinical interpretation of hypopnoeas.

Unfortunately, two definitions for hypopnoea were provided: the ‘recommended’ definition and the ‘alternative’. Ruehland et al. demonstrated that in the same sample, these definitions produce very different AHI values: for 328
diagnostic in-laboratory PSGs the median values were 25, 8 or 15 events/h for the Chicago, recommended or alternative criteria respectively. In 2012, the scoring rules were further updated. Note that, irrespective of the rules applied and technology used to collect the data, the cut-off values for AHI categories of nil, mild, moderate and severe OSA have been consistent.

2.5.6 **Heterogeneity in AHI definition**

There have been several sources of variation in AHI historically, which have made systematic comparison of prevalence and validation studies difficult (Section 2.6.1: Prevalence). Apnoea hypopnoea index is subject to substantial inter-laboratory variation due to differences in: i) scoring methodology (multiple definitions of apnoea and hypopnoea), ii) measurement technique (different equipment, sensors and number of channels recorded, Section 2.5.8), and iii) scorer variation in interpretation of events. Effort to standardise these three domains continues, with progressive evolution of measurement and analysis methods for the diagnosis of OSA. Without standardisation of measurement technique, scoring methodology and quality assurance for scorer reliability, it is difficult to make valid comparisons between studies or to build the databases required to advance our knowledge. In addition to the issue of standardisation for measurement and scoring, recent attention has focussed on the role of portable monitor testing in the diagnosis of OSA. Several reviews have examined current diagnostic technologies, and found major deficiencies in the evidence to support portable monitoring as a primary diagnostic tool. In a study described in Chapter 6 many of the deficiencies of past validation studies of Type 4 portable
monitoring (PM) devices are addressed, providing stronger evidence in support of a role for home PM testing in the clinical diagnosis of OSA.

2.5.7 The evolution of portable monitor studies to diagnose OSA

The history of PMs for unattended home sleep testing dates back to 1981. Early approaches involved oximetry alone but more complex systems with multiple sensors soon followed. Thus simple PMs have been in use for many years, their use driven partially by factors such as ease of access and cost compared with full laboratory-based PSG. There has been rapid growth in use of PM testing in clinical applications, and indeed this has become the mainstay approach in some settings. Despite the intuitive appeal for the use of PM testing to diagnose OSA, the evidence base to support its role in the clinical management of OSA patients is limited.

The first practice parameters for the use of portable recording in the assessment of OSA were published in 1994, prior to those for PSG. There is a wide spectrum of diagnostic methodologies available for the diagnosis of OSA, ranging from laboratory-based attended studies (PSG) with multiple recording channels to single or dual channel home-based studies where only oxygen saturation or respiratory airflow may be monitored. The development of these simpler technologies has been driven predominantly by a steady increase in pressure to identify OSA in the community over the past thirty years due to the increased prevalence of OSA (Section 2.6.1). The growing demand from patients to access diagnosis and treatment has resulted in increased wait times for diagnostic testing, creating pressure for simpler testing methods.
Faced with this pressure to diagnose and treat OSA, many physicians have resorted to the use of ambulatory approaches to diagnosis. In 2004 Flemons et al. reviewed the provision of sleep services in different countries. They reported that with increased clinical recognition of sleep apnoea, systems for delivering diagnosis and treatment are overwhelmed. Thus many services are using ambulatory approaches to diagnosis despite limited evidence to recommend their accuracy. In the UK, funding models have dictated that the first investigation for sleep-disordered breathing is usually a portable study, with two thirds of all studies comprising oximetry alone.

2.5.8 Types of portable monitor

The 1994 Practice Parameters described four levels of sleep study monitor ranging from full PSG (minimum 7 channels) to single channel (oximetry, 1 channel). The four levels are as follows: Type 1 is attended laboratory based PSG (up to 12 channels), Type 2 is portable PSG (≥ 7 channels), Type 3 is modified portable recording (usually 4-7 channels) and Type 4 is a continuous single or dual (1-2 channels) bioparameter recording. Subsequent reviews of portable monitors (PMs) have refined guidelines for the use of unattended PMs in the diagnosis of OSA in adults, but the four-level classification remains valid. Type 1 and 2 studies differ fundamentally from Type 3 and 4 studies in that sleep state is recorded during PSG (Type 1 or 2), whether the study is laboratory- or home-based. Thus, Type 3 or 4 PMs have fewer channels and do not record sleep state. For those sleep devices AHI calculation is based upon total recording time rather than total sleep time. To differentiate the index calculated for a portable study from that of PSG, the naming convention of respiratory disturbance index
(RDI) was commonly used. Overall this tends to result in an underestimation of AHI since time awake cannot be accounted for. Most recently the term RDI has been used to define the sum of the AHI and respiratory effort-related arousal events (RERAs) in studies where EEG is recorded. Thus the literature can be confusing and care must be taken to clarify the intended definition for RDI when comparing indices.

Despite this limitation, PMs have continued to gain acceptance to ‘rule in’ OSA. Where there is a high pre-test probability of moderate to severe OSA many PMs have been shown to be accurate for ruling in the disorder, with acceptable sensitivity and specificity optimising true positives and minimising false negatives. Current AASM clinical guidelines (US) for the evaluation of OSA demand objective testing with either laboratory-based PSG or home testing with PMs, but specific parameters must be measured. Portable monitor testing can be used as part of a comprehensive sleep evaluation in patients with a high pre-test likelihood of moderate to severe OSA but not in patients with major comorbid conditions. Current guidelines for the use of home sleep testing vary internationally.

2.5.9 Validation of portable monitors

There has been a concerted effort to evaluate the efficacy of PMs over the past twenty years in an attempt to improve access to OSA diagnosis and treatment. This approach might decrease cost and increase patient access, leading to earlier diagnosis and faster initiation of treatment, and decrease the healthcare burden of OSA (further described in Section 2.10.4). Although practice parameters were published in 1994, the first systematic review found major deficiencies in
empirical evidence to support the role of PM studies in the clinical management of OSA. The predominant shortcomings in validation of PMs included failure to evaluate their effectiveness in their intended home setting, lack of randomisation of order of at-home and in-laboratory studies, low sample size, and reliance on automated scoring of the data generated.43

An evidence grading system was developed so that future studies would use appropriate research methods with minimal bias.43,128 Subsequent reviews have used this grading methodology to build a valid evidence base to establish the role of PMs in OSA diagnosis and treatment.45,116 In Australia, Medicare has approved the use of Type 2 PMs for at-home or unattended sleep testing.129 This decision was based on evidence available from the Sleep Heart Health Study (SHHS) where it was shown that Type 2 studies can reliably rule-in and rule-out OSA.130,131 In 2008, Type 3 PMs were approved for home testing in the US.132 Two studies had demonstrated that the mode of testing (PM study versus laboratory PSG) did not impact the patient’s use of therapy.133,134 In the US, the most recent document regarding research priorities128 concluded that the most important need is to conduct adequately powered, good quality studies to generate the evidence required to continue the effort to build ambulatory management into current practice.

A major advantage of Type 3 and Type 4 PMs is that sensors can be self-applied by the patient at home. However acceptance of these simple PMs has been hampered by their lack of an accurate measure of time asleep and their inability to detect arousals, and therefore arousal-related events. Some clinicians have argued that it is critical that PMs accurately categorise presence or absence of
OSA, more so than exact agreement with PSG.\textsuperscript{135} Thus Collop et al. have devised a strategy to ensure PM diagnosis accurately categorises OSA, by addressing both study quality and application of a statistical methodology to confidently rule in OSA.\textsuperscript{45}

Despite progress, the AASM holds that there is insufficient evidence to support use of Type 4 PMs in unattended settings.\textsuperscript{116,127} Oximetry is the cornerstone signal of sleep apnoea monitoring\textsuperscript{129} and has been shown to accurately diagnose moderate to severe OSA in a population with a high pre-test probability when combined with questionnaires and a thorough history and examination.\textsuperscript{136} However the key is to apply the PM to the appropriate population in the correct situation.\textsuperscript{121} The Australasian Sleep Association (ASA) made the same conclusion with regard to Type 4 PMs, and thus further work is required to definitively determine their place in diagnostic testing, for both clinical and research purposes.\textsuperscript{129} Chapter 6 addresses the gap in the knowledge base for simple home PMs by comprehensively evaluating a two-channel monitor to 'rule-in' OSA in patients with a high pre-test probability of disease.

2.6 Epidemiology of OSA

Epidemiological studies of OSA have revealed a high prevalence of sleep-disordered breathing in the general community,\textsuperscript{137} presenting a significant public health problem.\textsuperscript{36,138,139} Although the clinical features of OSA were described in 1976,\textsuperscript{101} there was limited attention outside the field of sleep medicine. Most of the cases identified in the early years were symptomatic patients with moderate to severe disease referred to sleep clinics. However, population-based epidemiologic
studies have found a high prevalence of undiagnosed OSA (Section 2.6.1), with the severity ranging widely from simple snoring to severe OSA.

Of great concern is the large percentage of individuals suffering from mild OSA with or without overt daytime symptoms, since even mild OSA is associated with significant morbidity. There is strong evidence for an association of OSA with cardiovascular and cerebrovascular morbidity and mortality, as well as adverse public health consequences due to cognitive impairment. Many of the neurobehavioural deficits associated with OSA (such as reduced attention, concentration, vigilance, manual dexterity and visual motor skills) have important impacts on driving ability and as such have been extensively investigated. Since traffic safety is under governmental regulation, there are legal implications for both private and commercial drivers if OSA is a significant cause of impaired driving (Section 2.9). Chapter 3 addresses the question of whether OSA increases the risk of motor vehicle crashes and which morbidities associated with OSA predict increased risk.

### 2.6.1 Prevalence

In 1993, Young et al. reported that 2% of women and 4% of men have OSAHS, and 9% of women and 24% of men have OSA. These data from the Wisconsin Sleep Cohort Study (WSCS) have been verified in methodologically similar large sample studies in the USA and Spain. Most striking was the size of the problem and the proportion of people with OSA but without overt symptoms. There was concern about this large undiagnosed population in the community. Although the clinical significance of OSA without overt symptoms is controversial, many studies have shown adverse health outcomes are
associated with OSA regardless of the presence of excessive daytime sleepiness.\textsuperscript{36,145}

\subsection{2.6.2 Risk factors}

Sex, age and obesity are important risk factors for OSA, with physical inactivity, smoking, and alcohol consumption increasing the occurrence of the disorder.\textsuperscript{138} Each of these demographic and lifestyle risk factors has a potential role in mediating the presence and severity of OSA and must be considered as confounders in their association with OSA.

\subsubsection{2.6.2.1 Sex}

Most studies have consistently reported that there is a two to three fold greater risk of OSA in men compared with women.\textsuperscript{93} The reason for the male predominance is not exactly clear, but sex hormones may play a role, since the prevalence is higher in post-menopausal females.\textsuperscript{146} Other explanations include sex differences in body fat distribution, differences in pharyngeal anatomy and function, and hormonal influences affecting muscles of the upper airway and its ability to collapse.\textsuperscript{147} The gender difference has relevance to public health in that many more men than women are occupational drivers.\textsuperscript{148}

\subsubsection{2.6.2.2 Age}

The prevalence of OSA increases with age, with some studies showing a doubling of AHI every ten years independent of a rising body mass index (BMI) with age.\textsuperscript{149} The peak age of presentation is about fifty years with the prevalence falling thereafter. Data from the SHHS suggest that OSA remains prevalent in the elderly, but more often without symptoms.\textsuperscript{150}
2.6.2.3 Obesity

Obesity is a commonly reported risk factor for OSA that predisposes to and potentiates OSA.\textsuperscript{152} There is a lack of controlled studies that evaluate the effect of weight loss with conservative therapy, but longitudinal data from the WSCS found a 10% weight gain predicted a 32% increase in AHI. By contrast a 10% weight loss predicted a 26% decrease in AHI.\textsuperscript{151} More recent studies on patients pre- and post-bariatric surgery have provided useful data on the effect of more dramatic weight loss.\textsuperscript{152} A review by Romero-Corral \textit{et al.} found that overall patients undergoing bariatric surgery had a consistent reduction in weight and AHI, whereby every 1% weight reduction in BMI translated to a decrease of 2.3% in AHI.\textsuperscript{152} Thus obesity has a direct effect on the severity of OSA, but is stronger in men than women.\textsuperscript{153} Body mass index (BMI) is the index of obesity most often used, but controversy remains as to whether either neck or waist circumference may better predict sleep-disordered breathing. Nevertheless, clinical epidemiologic studies frequently use BMI to control for the confounding influence of obesity on OSA outcomes. A recent study designed to explore the natural history of excessive daytime sleepiness in a population-based cohort has found that obesity is a major risk factor for the incidence and chronicity of excessive daytime sleepiness, independent of the presence of OSA.\textsuperscript{154} These data underscore the importance of controlling for the confounding effect of obesity with respect to association with OSA and excessive daytime sleepiness.
2.6.2.4 Physical inactivity

Less research has been done on the role of physical inactivity on OSA, but there has been increased interest in relation to the obesity epidemic. Findings from the WSCS suggest that physical inactivity is associated with sleep-disordered breathing after controlling for BMI.\textsuperscript{155} There have been cohort studies in both Scandinavia\textsuperscript{156} and the US\textsuperscript{157} examining the relationship between physical inactivity and snoring, but the results have been contradictory.\textsuperscript{139} The impact of physical inactivity on OSA remains unclear, although a recent Australian study reported that low levels of physical activity were associated with moderate to severe OSA.\textsuperscript{158}

2.6.2.5 Smoking

Few studies have been conducted specifically to investigate the association between smoking and OSA. However one study found that current smokers were three times more likely to have OSA than never-smokers.\textsuperscript{159} The proposed mechanisms whereby smoking predisposes the airway to collapse include airway inflammation and smoking-related sleep instability mediated by nicotine withdrawal. Several cross-sectional epidemiological studies\textsuperscript{157,160,161} have found a positive association between smoking and either snoring or OSA, but strangely, the Sleep Heart Health Study (SHHS) found that smokers had less OSA than non-smokers.\textsuperscript{162} Thus although there is biological plausibility for a causal role of smoking in OSA, it is not firmly established as a risk factor.
2.6.2.6 Alcohol

Two types of study have analysed the effect of alcohol on OSA. Experimental studies show that alcohol intake reduces motor output to the upper airway, exacerbating collapse during sleep.\(^{163}\) Findings from epidemiological studies have been inconsistent, with the association between self-reported alcohol consumption and OSA unclear and the effect of long-term alcohol use on the occurrence of OSA unknown.\(^{36}\)

In summary, OSA is a prevalent condition with many undiagnosed cases in the community. There are inadequate diagnostic facilities to manage the burden of disease. Consequently there is a need for improvement in disease detection and categorisation so that individuals with undiagnosed OSA at greatest risk of motor vehicle crashes or work-related accidents are promptly identified. This public health issue is addressed in both Chapters 3 and 5 of this thesis.

2.7 DAYTIME SLEEPINESS

2.7.1 Descriptors for daytime sleepiness

Many different terms have been used to describe the phenomenon of sleepiness in the scientific literature. The two most common are ‘excessive sleepiness’ and ‘fatigue’. Multiple terms have arisen because not all sleepy individuals describe their symptoms as sleepiness \emph{per se}, but rather as a consequence of sleep loss such as fatigue, loss of energy, lethargy, weariness, memory lapses, lack of initiative, or difficulty concentrating.\(^{164-166}\) In sleep medicine the term excessive sleepiness is defined as a heightened propensity to fall asleep while involved in activities that require alertness,\(^{83,167}\)
Certain terminology is favoured in specific research domains, such as occupational health and safety. With respect to the link between fatigue and safety, a recent literature review favoured the term fatigue, simply defined as “a biological drive for recuperative rest”\(^{168}\). However, fatigue may take several forms including sleepiness and mental, physical and/or muscular fatigue depending on the nature of its cause\(^{169}\). In 2005 Philip \textit{et al.} presented a distinction between fatigue and sleepiness. Fatigue increases with sustained activity and can be eliminated with rest but not necessarily sleep, whilst sleepiness is the subjective need to sleep that cannot be eliminated through rest from activity alone\(^{169}\). Thus sleep is required to alleviate sleepiness, but will not necessarily relieve fatigue. In this thesis the term excessive sleepiness is used to define the physiological problem of disabling sleepiness both in relation to sleep loss (volitional) and the sleep disruption due to the medical condition of OSAHS.

### 2.7.2 Excessive daytime sleepiness and OSA

The most common daytime symptom in OSA is excessive daytime sleepiness\(^{100}\). OSA is the second most common cause of excessive daytime sleepiness aside from insufficient sleep, but not all OSA subjects report this symptom\(^{83}\). Among subjects in the SHHS, less than 50% of those with moderate to severe OSA reported excessive daytime sleepiness\(^{19}\). There is a poor correlation between sleepiness symptoms and OSA severity (as measured by the AHI), with the correlation rarely rising above an \(r\) value of 0.4\(^{149}\). This poor correlation is partially due to variability of measurement techniques and scoring rules between laboratories (Section 2.5.6) and the difficulty of measuring sleepiness accurately (Section 2.7.3). Thus studies have shown that OSA can be present without overt
sleepiness, and symptoms may be represented by another ‘fatigue’ descriptor (Section 2.7.1).11,170

In adults, excessive daytime sleepiness may cause or exacerbate mood disturbance (including irritability, fatigue, depression, anxiety) and cognitive deficits (including attention and vigilance, memory and executive functions, and simulated driving and motor vehicle crashes), resulting in occupational and family problems and reduced quality of life.20 Excessive daytime sleepiness is often under-recognised in the general population and even in specific sleep disorders because subjects do not complain about it or recognise that it is present.171 Some recognise that daytime sleepiness is present but feel that it does not impact on their quality of life, and sometimes physicians fail to inquire about this symptom.83

It has long been recognised that chronic excessive daytime sleepiness can be ignored by subjects simply because of an inability to distinguish normal from abnormal.171 There is a phenomenon of under-reported sleepiness in OSA subjects (especially prior to treatment) first noted by Dement in 1981.172 This has been attributed to a loss of the normal frame of reference for alertness as a result of prolonged excessive sleepiness. It has been postulated that the internal calibration of feeling ‘refreshed’ was gradually reset over many years of untreated OSA.166,172 This is particularly evident in cases where excessive daytime sleepiness is denied at presentation, but a successful trial of treatment resets the baseline of refreshing sleep and perception improves.166,171 Further evidence of adaptation to chronic sleepiness in OSA is found in the results from laboratory-based sleep deprivation studies in healthy subjects. After one night of sleep deprivation alert individuals were able to accurately estimate their sleepiness, in contrast to OSA subjects.173
2.7.3 **Assessment of sleepiness**

Two issues hamper accurate evaluation of excessive daytime sleepiness. The first relates to the description of the symptom by the patient, which may be inaccurate, and the second is the inability to measure it readily. There are several subjective sleepiness scales and objective tests available, but all have limitations. The most important tool is a carefully taken clinical history from the patient, along with collateral history from a bed partner or relatives.92

2.7.3.1 **Clinical history**

In order to assess the presence of sleepiness associated with OSA, details of the patient’s typical night sleep time and sleep-wake schedule are important, along with other typical symptoms of snoring and witnessed apnoea. The degree and relevance of excessive daytime sleepiness should be clinically evaluated, with special emphasis on driving performance. It is important to differentiate true sleepiness (the urge to sleep) from various forms of tiredness such as lethargy, poor concentration or malaise.109 To help confirm the presence of the symptom of excessive daytime sleepiness, tools which measure subjective and objective sleepiness can be used.

2.7.3.2 **Epworth sleepiness scale**

The most widely used subjective sleepiness scale in clinical practice is the Epworth Sleepiness Scale (ESS).88 It was developed to measure the tendency to fall asleep in several specific situations encountered in normal daily life. The ESS is a specialised, validated sleep questionnaire with eight items asking self-report of the likelihood of ‘dozing’ (on a scale of 0-3) in various situations (Table 2.3). The scale
ratings are added to give an ESS score, from 0 to 24. An ESS score greater than 10 represents excessive daytime sleepiness.88

Table 2.3. The Epworth Sleepiness Scale,88 a self-report of the likelihood of ‘dozing’ in various daily situations.

<table>
<thead>
<tr>
<th>Question</th>
<th>Situation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sitting and reading</td>
</tr>
<tr>
<td>2</td>
<td>Watching television</td>
</tr>
<tr>
<td>3</td>
<td>Sitting and inactive in a public place (e.g., a theatre or a meeting)</td>
</tr>
<tr>
<td>4</td>
<td>As a passenger in a car for an hour without a break</td>
</tr>
<tr>
<td>5</td>
<td>Lying down to rest in the afternoon when circumstances permit</td>
</tr>
<tr>
<td>6</td>
<td>Sitting and talking to someone</td>
</tr>
<tr>
<td>7</td>
<td>Sitting quietly after lunch without alcohol</td>
</tr>
<tr>
<td>8</td>
<td>In a car, while stopped for a few minutes in traffic</td>
</tr>
</tbody>
</table>

Recently the ESS construct has been considered further to explore the concept of sleep propensity and somnificity.174-176 The eight questions can be grouped according to how soporific (i.e. sleep inducing) the situation is, considering both the posture of the subject and the environmental circumstances. For example, question 5 (Lying down to rest in the afternoon when circumstances permit) is the most soporific situation but many individuals do not have the opportunity to do so. By contrast, question 8 (In a car, while stopped for a few minutes in traffic) should describe an alerting situation for most people, so that any indication of the possibility of dozing is alarming. Researchers have increasingly explored use of this concept of somnificity to identify individuals at risk of dozing in potentially dangerous situations such as driving.176,177
Support for the ESS as a valid subjective sleepiness tool has been shown by a small but statistically significant correlation with the multiple sleep latency test (MSLT), an objective index of sleep drive or propensity. The ESS has gained wide acceptance due to its ease and practicality of use, low cost and high test-retest reliability. Drawbacks include poor correlation with the severity of OSA and the usual problems associated with self-evaluated tests of misperception of sleep episodes and cheating.

2.7.3.3 Objective measures of sleepiness

Objective tests which give a physiological measure of sleepiness have obvious advantages over subjective sleepiness scales, but are time-consuming and may not reflect every day activity. The MSLT, which identifies sleep tendency by measuring the time it takes to fall asleep on repeated occasions at two-hour intervals during the day, is considered by experts to be the most reliable objective test for the measurement of excessive daytime sleepiness.

A variant of the MSLT is the maintenance of wakefulness test (MWT), which is generally used to assess the efficacy of a treatment after diagnosis. This test measures the ability of the patient to remain awake and so may be a more relevant test to assess driving ability. No measurement tools exist to test for sleepiness at a motor vehicle crash site in the same way as for alcohol. However some monitoring devices designed to assess drowsiness have been developed for use in safety-critical industries and these have potential for real-time management of sleepiness-related risk in the workplace. Better still however would be strategies that minimise the likelihood that people will be sleepy in such workplaces, since detection alone is not sufficient.
2.7.4  The cause of sleepiness in OSA

What causes the sleepiness associated with OSA? Two primary physiological pathways have been proposed, but the pathophysiological mechanisms for the neurobehavioural impairment in OSA are unclear.\textsuperscript{182} The presence of excessive daytime sleepiness in patients with OSA has been ascribed to fragmentation of sleep,\textsuperscript{183,184} to the intermittent hypoxia associated with breathing pauses,\textsuperscript{185} or both.\textsuperscript{186-188}

2.7.4.1  Sleep Fragmentation

OSA causes breathing disturbances which fragment sleep and hence disrupt its restorative process.\textsuperscript{189} The degree of fragmentation is variable, ranging from the least disruption due to repeated micro-arousals (of 3 seconds duration), to brief awakenings with shifts to a lighter stage of sleep, and finally to altered sleep architecture with a reduction or excess of different stages of sleep (such as reduced REM sleep or SWS).\textsuperscript{190} Patients are usually unaware of this sleep fragmentation, since they are not overtly waking, but can report feeling unrefreshed upon waking in the morning. There have been three reviews of the large body of work devoted to experimental sleep fragmentation, with some contradiction as to the conclusion that sleep fragmentation leads to sleepiness.\textsuperscript{190-192}

In 2003, Bonnet \textit{et al.} reported that sleep fragmentation shortened sleep latency and increased subjective sleepiness proportionally to the frequency of sleep fragmentation.\textsuperscript{191} This was a consistent finding when change in MSLT was
plotted as a function of rate of sleep fragmentation for a group of eight studies.\textsuperscript{191} Several studies have carefully controlled the sleep fragmentation to maintain all nocturnal sleep stage parameters and still shown significant increases in objective sleepiness.\textsuperscript{91,193} In a carefully designed series of animal experiments, Phillipson \textit{et al.} demonstrated that sleep fragmentation resulted in increased sleepiness and impaired arousal response in dogs.\textsuperscript{194} Thus there is evidence that disturbance of sleep continuity, rather than changes in sleep stage, produces the effects of sleep fragmentation.\textsuperscript{191}

A review by Reynolds and Banks (2010) concluded that, overall, the evidence from sleep fragmentation studies confirmed an association with both objective and subjective sleepiness.\textsuperscript{192} More recently, Aloia and Arnedt (2012) concluded that there was a linear relationship between increasing rate of fragmentation and increased objective sleepiness in eight of fourteen studies.\textsuperscript{190} Consideration of this body of animal and human research confirms that sleep fragmentation can lead to daytime sequelae, but not all studies demonstrate this relationship by the induction of sleep fragmentation in healthy controls.\textsuperscript{190}

Note that sleep fragmentation differs from sleep deprivation in terms of neurocognitive outcomes,\textsuperscript{191,195} and few studies have made direct comparisons. However there is a large body of literature devoted to sleep deprivation studies, including total sleep deprivation and chronic partial sleep deprivation in healthy controls, that shows behavioural, psychological and physiological consequences that impair function.\textsuperscript{191,195,196} Chronic partial sleep deprivation (which may be comparable to the sleep loss seen in OSA) causes significant increases in subjective sleepiness, and decreases in mood and vigilance.\textsuperscript{197} The neurocognitive
consequences of sleep deprivation are reviewed in Section 2.8 and compared with those of OSA in Section 2.8.6.

2.7.4.2 Intermittent hypoxia

Intermittent hypoxia is common in OSA but not universal, and can lead to the development of cognitive dysfunction. Daytime impairment in OSA patients has been suspected to be a consequence of either brain dysfunction and/or damage, with intermittent hypoxia a likely contributor. Breathing pauses (apnoea and hypopnoea) can cause intermittent hypoxia (defined as a desaturation of 3-4%), which leads to a disruption in the biochemical and haemodynamic state of the central nervous system. In the last ten years, clinical and experimental evidence suggests that exposure to prolonged periods of intermittent hypoxia may play a critical role in the development of cognitive morbidity. The major neurocognitive deficits are excessive daytime sleepiness, mood disturbances and impaired cognition.

The mechanism for these deficits may be structural neuronal damage induced by the intermittent hypoxia associated with OSA. In a rat model of OSA, Gozal et al. found that intermittent hypoxia caused neuronal death and was associated with impaired learning and memory. Further studies in rats and mice have suggested that exposure to hypoxia leads to injury in the wake-promoting brain regions, giving a potential mechanism for excessive sleepiness. Thus neural cell loss associated with intermittent hypoxia may underlie memory impairment in OSA in humans. This notion is supported by work comparing hypoxic to non-hypoxic OSA patients, where deficits in attention, vigilance and memory were associated with intermittent hypoxia.
In humans, Colt et al. conducted an experiment to attempt to differentiate the effects of sleep fragmentation from intermittent hypoxia in producing the excessive daytime sleepiness associated with OSA. They found that when OSA was treated with CPAP to reduce sleep fragmentation, and periods of desaturation were introduced experimentally, sleepiness did not return from periods of intermittent hypoxia alone. More recently, neuroimaging studies have shown that OSA patients are at increased risk of white and grey matter loss, alterations in markers of neuronal integrity and changes in prefrontal lobe vascular perfusion, but not all studies have replicated these findings.

In summary, both sleep fragmentation and intermittent hypoxia have been implicated as mechanisms leading to the excessive daytime sleepiness associated with OSA. It seems intuitive that sleepiness would be a direct result of the sleep fragmentation associated with breathing pauses and hypoxia, suggesting that fragmentation and intermittent hypoxia play a combined role. However a correlation between the daytime symptoms and the degree of AHI or hypoxaemia is not always seen, and there is not a consistent relationship between excessive daytime sleepiness and sleep fragmentation. Thus the pathophysiological mechanisms for the neurobehavioural impairment in OSA remain unclear, with both sleep fragmentation and nocturnal hypoxaemia implicated.
2.8 Total sleep deprivation, chronic sleep restriction and sleep fragmentation

Sleep deprivation and sleep disorders have gained considerable attention over the past ten years as a public health issue. There has been recognition of an increased prevalence of both insufficient sleep in adults (estimated at 20%),\textsuperscript{207} and OSA, the most common sleep disorder (2 and 4% of adults)\textsuperscript{36} causing excessive daytime sleepiness. Sleep loss results in impairment in both cognitive performance and simulated driving, and induces sleepiness, fatigue and mood changes.\textsuperscript{192,195} Total sleep deprivation has been widely researched, with the results providing useful insight into the detrimental effects of night shift work.

More recently, there has been increased focus on chronic sleep restriction and fragmentation due to widespread concern about the pervasive negative effect of chronic sleep loss in society,\textsuperscript{53,59} as a result of medical conditions, sleep disorders (especially OSA) and lifestyle factors (e.g. shift work, jet lag and prolonged work hours). Due to recent more rigorously designed studies, it is now understood that chronic sleep loss has a similar negative impact on cognitive function as a period of total sleep deprivation.\textsuperscript{192} Following is a summary of the neurocognitive consequences of total sleep deprivation and chronic sleep restriction, and a comparison between sleep fragmentation (as seen in OSA) and sleep deprivation. Review of this laboratory-based work (in both healthy and OSA subjects) will provide insight into the relationship between neurocognitive impairment in OSA and accident risk. Sleep deprivation studies have most commonly used three measurement categories of neurocognitive function: cognitive performance, mood and motor performance.\textsuperscript{196}
2.8.1 Total sleep deprivation

Many individuals regularly experience total sleep deprivation as part of shift work (e.g. truck drivers, nurses, pilots, mine workers) and so an accurate understanding of the effects of sleep deprivation on cognitive function is critical for safety.\textsuperscript{59,208} Total sleep deprivation is defined as the elimination of sleep for a period of time (at least one night). Total sleep deprivation has served as an experimental methodology to develop our understanding of the function of sleep. Early experiments found that memory and reaction time were significantly affected by total sleep deprivation, and hundreds more experiments have explored its behavioural and physiological effects.\textsuperscript{195} Overall, total sleep deprivation has a negative impact on four domains of cognition and daytime function: (i) mood, (ii) cognitive performance - psychomotor and cognitive speed, working memory and higher cognitive abilities, (iii) motor function - vigilant and executive attention, and (iv) sleep propensity - as reflected in both objective and subjective measures of sleepiness.

2.8.1.1 Cognition and total sleep deprivation

Most cognitive performance measures show decrements with total sleep deprivation (Table 2.4) and, regardless of the task, performance becomes progressively worse with increased time on task. The psychomotor vigilance test is one of the most sensitive cognitive assays to sleep loss,\textsuperscript{209} is an objective measure of vigilant attention, and requires continuous attention to detect randomly occurring stimuli. It reliably shows changes in reaction time with changes in sleep opportunity and has been widely used in total sleep deprivation studies. The psychomotor vigilance test is free of aptitude and learning effects and sensitive to
sleep loss, sleep pathology and functioning at an adverse circadian phase. Studies have consistently found decrements in vigilant attention with total sleep deprivation, with clear implications for increased risk of errors in operational settings. This is reflected in the psychomotor vigilance test as increased errors of both omission (failure to respond) and commission (response when not required). Table 2.4 gives a summary of the cognitive performance effects of sleep deprivation. Note that these tasks vary considerably in their sensitivity to sleep loss, and part of this variability may be attributable to intra- and inter-subject variability (Section 2.8.5).
Table 2.4. Summary of cognitive performance effects of total sleep deprivation\textsuperscript{195}

<table>
<thead>
<tr>
<th>Effect</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Involuntary microsleeps occur</td>
<td></td>
</tr>
<tr>
<td>Attention-intensive performance is unstable with increased errors</td>
<td>Increased errors of omission (lapses) and commission (responses)</td>
</tr>
<tr>
<td>Cognitive slowing occurs in subject-paced tasks, whereas time pressure increases cognitive errors</td>
<td></td>
</tr>
<tr>
<td>Psychomotor response time slows</td>
<td></td>
</tr>
<tr>
<td>Both short-term recall and working memory performances decline</td>
<td></td>
</tr>
<tr>
<td>Reduced learning (acquisition) of cognitive tasks occurs</td>
<td></td>
</tr>
<tr>
<td>Performance requiring divergent thinking deteriorates</td>
<td></td>
</tr>
<tr>
<td>Response suppression errors in tasks primarily subserved by the pre-frontal cortex</td>
<td></td>
</tr>
<tr>
<td>Response perseveration on ineffective solutions is more likely to occur</td>
<td></td>
</tr>
<tr>
<td>Increased compensatory effort is required to remain behaviourally active</td>
<td></td>
</tr>
<tr>
<td>Tasks may begin well, but performance deteriorates as task duration increases</td>
<td></td>
</tr>
<tr>
<td>Growing neglect of activities judged to be non-essential (loss of situational awareness) occurs</td>
<td></td>
</tr>
</tbody>
</table>

Most recently, advances in technology (functional magnetic resonance imaging) have allowed insight into the brain changes associated with total sleep deprivation. This work lends support to the hypothesis that the frontal region of the brain is crucial for high level cognitive function and that total sleep deprivation results in decreased activation and reduced performance.\textsuperscript{212}
2.8.1.2 Sleepiness and total sleep deprivation

Sleep latency as measured by objective tests (MSLT and MWT) is decreased with total sleep deprivation. Thus sleep propensity is inversely related to the duration of time awake. Increased sleep propensity can manifest as physiological intrusions of sleep into wakefulness. Examples are sleep attacks (i.e. involuntary naps), microsleeps, slow eyelid closures and slow rolling eye movements, all of which can be measured and represent periods of reduced vigilance. It is likely that the intrusion of “microsleeps” into wakefulness is the cause of the lapses seen in the psychomotor vigilance test in sleep-deprived individuals. Subjective measures such as fatigue, sleepiness and mood are also affected by a period of total sleep deprivation. Since virtually all studies of total sleep deprivation have found alterations in subjective sleepiness, simple self-report excessive daytime sleepiness tools such as the ESS can be used to monitor changes in excessive daytime sleepiness pre- and post-treatment.

2.8.1.3 Accidents, alcohol and total sleep deprivation

By necessity, most total sleep deprivation work has been conducted in the tightly controlled laboratory environment in order to better understand the real-world implications of sleep loss. There is a demonstrated link between total sleep deprivation and traffic and workplace incidents. Much research into total sleep deprivation is directed toward an understanding of the effects of shift work since extended wakefulness occurs at the start and end of a period of night shift in particular. Examples of increased accidents in association with extended wakefulness have been found in safety-sensitive industries such as medicine and aviation.
Studies comparing the effects of alcohol intoxication with total sleep deprivation have found equivalent performance impairment. Dawson and Reid showed that after 17 hours of wakefulness cognitive psychomotor performance decreased to the same level of impairment seen at a blood alcohol concentration of 0.05%. Note that this alcohol/time awake comparison only applies for an eight in the morning wakeup time, and the impact of time awake on performance varies with circadian phase. Nevertheless, this observation has been useful since it provides an easily-grasped index of the relative impairment associated with sleep loss. Since many western industrialised countries regulate the level of blood alcohol for driving, this direct comparison with a modest level of sleepiness helps the community appreciate the consequences of sleep loss. The combined effects of alcohol and total sleep deprivation have been studied with simulated driving protocols. Overall this work suggests that the combination of moderate total sleep deprivation and legal amounts of alcohol consumption has an additive effect that significantly increases driving accident risk.

2.8.2 Partial sleep deprivation

Chronic sleep restriction is common in society but comparatively little research has been done relative to total sleep deprivation. Total sleep deprivation is representative of the sleep loss seen in shift workers and affects about 20% of the population. Chronic partial sleep deprivation is a pervasive phenomenon in the community, with an equivalent proportion (20%) of adults reporting insufficient sleep. Sleep debt is a global term that describes the accumulating effects of inadequate sleep. This may be due to insufficient duration and/or poor quality sleep caused by; the adverse effects of untreated sleep disorders, the
consequences of night-shift work and jet lag and the effects of sleep restriction related to social and work responsibilities. Thus sleep debt encompasses both total and partial sleep deprivation, with research in both domains central to our overall understanding of the impact of sleep loss on performance and health.

There are several terms used in the literature to describe partial sleep deprivation, including chronic or partial sleep deprivation, sleep restriction, sleep loss and insufficient sleep. In this thesis, the term chronic partial sleep deprivation is used to define research done in healthy individuals whereby time in bed (i.e. sleep opportunity) is restricted to 4 to 7 hours. Partial sleep deprivation occurs when sleep is reduced below an individual’s usual baseline (Section 2.4.1). The most relevant working definition for basal sleep requirement is the amount of sleep needed on a regular basis to maintain optimal performance.

Early research suggested there were no detrimental effects of chronic partial sleep deprivation (sleep restricted to 4 to 6 hours time in bed per night for up to 8 months) on neurocognitive function. However these studies were not well controlled and over the past fifteen years there has been increased focus on the methodological rigour of sleep restriction protocols. Recent studies conducted in a controlled laboratory setting have shown significant impairment of cognitive function and changes in sleep propensity, subjective sleepiness, mood and risk of motor vehicle crashes.

2.8.2.1 Cognitive deficits arising from partial sleep deprivation

There have been several recent well-controlled studies of partial sleep deprivation that have found systematic deterioration in cognitive function across
days when sleep duration is chronically restricted. Specifically, subjects experiencing four or more consecutive nights of less than 7 hours sleep showed cumulative effects on their daytime performance as measured by the psychomotor vigilance test. The most extensive, controlled dose-response experiment on partial sleep deprivation to date compared 14 days of sleep limitation (4, 6 or 8 hours sleep) with 1 to 3 nights of total sleep deprivation. Cognitive and vigilance tasks were done two hourly, along with subjective sleepiness and continuous EEG recording. Three days of total sleep deprivation resulted in much larger deficits than any of the three partial sleep deprivation conditions. However 14 days of 4 to 6 hours sleep produced equivalent cognitive deficits to 24 to 48 hours of total sleep deprivation.

2.8.2.2 Simulated driving and partial sleep deprivation

Decreased driving ability is a real-world risk associated with partial sleep deprivation. As little as one night of sleep reduced to 5 hours can impair simulated driving performance. Similar impairment has been found in studies of more than one night of sleep restriction independent of the timing of the sleep opportunity.

2.8.2.3 Excessive sleepiness and partial sleep deprivation

Carskadon and Dement found objective increases in sleepiness with decreased sleep latencies on MSLT across 7 nights of 5 hours sleep per night, supporting the subjective sleepiness findings. In two studies, the data for subjective sleepiness showed an immediate elevation, but this response plateaued and did not show the cumulative increases that were found in cognitive measures. Although the subjective sleepiness measures were elevated with
both partial and total sleep deprivation, they did not predict the degree of performance impairment, and tended to underestimate the degree of cognitive dysfunction induced by the sleep loss.\textsuperscript{61} Thus individuals seem less able to accurately report their sleepiness while showing increasing deficits in performance.\textsuperscript{192}

2.8.3 Sleep fragmentation

The high incidence of fragmenting sleep disorders, especially OSA, has drawn increased attention to the problem of sleep disruption. Sleep fragmentation has not been as heavily researched as sleep deprivation, but a number of studies have shown that disrupted sleep has significant consequences for cognitive function\textsuperscript{191}(Section 2.7.4.1). The methodology used to fragment sleep varies between studies, and can itself change the structure and duration of sleep. Studies have disrupted sleep in the following ways: i) at a specific sleep stage (e.g. fragmentation of SWS or REM sleep), ii) sleep fragmented with auditory tones to mimic a sleep disorder, and iii) sleep fragmented to awakening, thus altering sleep architecture and total sleep time. Individuals with fragmented sleep can maintain a normal sleep duration, and thus the mechanism of reduced alertness and impaired cognition is distinctly different to that of sleep deprivation.\textsuperscript{192} However, sleep fragmentation has substantial effects upon cognition and symptoms of sleepiness.

2.8.3.1 Sleep fragmentation, cognition and sleepiness

Most sleep fragmentation studies (13 out of 14) have found decreased psychomotor performance on days following disturbed sleep.\textsuperscript{191,192} Sleep fragmentation also affects working memory and cognitive speed. Several studies have carefully controlled total sleep time to confirm that the effects of sleep
fragmentation are not explained by partial sleep deprivation. In summary, sleep fragmentation paradigms have shown shortened sleep latency and increased subjective sleepiness that is proportional to the frequency of sleep fragmentation.

2.8.4 Comparison between total and partial sleep deprivation and sleep disruption

Sleep deprivation (total or partial) and sleep fragmentation all have significant negative effects on cognitive performance and sleepiness. High frequency sleep fragmentation (equivalent to severe OSA) has equivalent effects to total sleep deprivation. There was no significant difference in outcomes when sleep was fragmented every minute compared with total sleep deprivation for 64 hours. In another study, decrements in performance accumulated across 14 nights of 4 hours sleep were similar to 2 nights of total sleep deprivation.

However the rate of change in the development of cognitive deficits and sleepiness differed between the two conditions. With total sleep deprivation, cognitive decrements were paralleled by increased self-reported fatigue and sleepiness, whereas individuals subjected to partial sleep restriction lacked the ability to perceive their level of impairment. Ratings of sleepiness and mood tend to plateau after several days, while cognitive performance continues to deteriorate. This critical difference has potential implications for safety and risk-taking in the sense that individuals hampered by sleep restriction may be more inclined to drive while significantly impaired. Cognitive deficits associated with total sleep deprivation and sleep disruption have been compared with performance after alcohol consumption. Work which extends Dawson's original
finding showed that cognitive function after 21 hours of total sleep deprivation or a night of sleep disruption is comparable to performance with a blood alcohol level of 0.08%. Thus total sleep deprivation and sleep disruption are equally detrimental.

All forms of sleep deprivation and disruption induce a wide range of effects on cognitive function, but the cognitive tasks vary in their sensitivity to sleep loss. Tasks that are most sensitive to sleep deprivation are speed of cognitive throughput, working memory and attention. The psychomotor vigilance test has been widely used to accurately measure performance during sleep loss since it is free of aptitude and learning effects and sensitive to sleep loss, sleep pathology and functioning at an adverse circadian phase. There are two confounding factors that can obscure the effects of sleep loss on many cognitive tasks; inter-subject variability and intra-subject variability (Section 2.8.5). An appreciation of the degree of this variability is crucial since it can be so wide as to mask the effects of sleep loss on cognitive measures.

2.8.5 Individual variability in human sleep

Ten years ago, sleep deprivation studies comparing total and partial sleep deprivation demonstrated that there are robust systematic individual differences in susceptibility to performance impairment from sleep loss. These differences in susceptibility are postulated to be trait-like since repeated exposure to sleep loss in the same subjects yields consistent deficits. Van Dongen et al. studied performance impairment from sleep loss in a controlled laboratory study of 21 subjects over three exposures to 36 hours of total sleep deprivation. Every two hours, subjects underwent a battery of neurobehavioural performance tests
and subjective sleepiness measures. For each subject, the observations were averaged over the last 24 hours of sleep deprivation to quantify the level of impairment due to sleep loss.

The results showed that subjects differed substantially in their responses to sleep deprivation, whereas the responses were relatively stable within subjects across the two test periods. The proportion of variance in the data explained by systematic inter-individual variability, quantified by the intra-class correlation coefficient, showed that 67.5% to 92.2% of the variance in the data was explained by stable variations among individuals. This was not due to variations in sleep history. In the first two periods of total sleep deprivation, subjects were allowed to satiate sleep need with sleep extension (12 hours in bed for one week prior). In the final test period, sleep was restricted (6 hours in bed for one week prior). After the sleep restriction challenge, the magnitude of the individual differences overwhelmed the additional impairment induced by the sleep restriction. Thus the differences were highly replicable within subjects, robust to manipulation of sleep history and persisted when baseline differences were controlled.23

This set of observable individual characteristics in response to sleep loss likely constitutes a sleep phenotype (Section 4).24 Subsequent to these findings there has been an extensive search for reliable predictors of an individual vulnerability to sleep loss, including genetic determinants,229 neuroimaging and other domains,195 and biomarkers.230 This search has been driven by the important implications of vulnerability to sleep loss from a public health perspective, and the opportunity to improve safety, productivity and well-being.24,208
In summary, variability between individuals is considerable and thus sleep phenotype and multiple potential intrusions upon sleep quality must be factored into any assessment of sleepiness-related risk. The cognitive deficits associated with OSA have been heavily researched over the past twenty years. A comparative analysis of neurocognitive function in OSA relative to the body of work described above for sleep deprivation is given in Section 2.8.6.

2.8.6 Cognition and daytime function in OSA

Comprehensive reviews in this area have concluded that sleep fragmentation has the same effect as sleep deprivation on waking behaviour, and thus the deficits associated with OSA are similar to those found in sleep deprivation and fragmentation studies. A range of cognitive deficits has been studied and can be categorised into four primary domains: (i) excessive daytime sleepiness, (ii) reduced psychomotor vigilance, (iii) cognitive (executive) dysfunction, and (iv) impaired mood. Of these, the most likely to impact on performance in safety-sensitive tasks are excessive sleepiness and psychomotor vigilance, especially in relation to driving.

It must be noted that the pathophysiology of OSA differs fundamentally to the experimental sleep deprivation paradigms described in Sections 2.7.1 and 2.7.2. In OSA two nocturnal physiological abnormalities (hypoxaemia and sleep fragmentation) occur and it is likely that both contribute to cognitive daytime impairments (Section 2.7.4). Studies in humans have shown that sleep fragmentation is linked to deficits in sustained attention and excessive daytime sleepiness. Animal work by Gozal has implicated hypoxaemic neural damage in relation to deficits in executive function. Recent studies have also shown that
OSA creates a pro-inflammatory state that may contribute to neurocognitive dysfunction, OSA-related arousals are associated with increases in autonomic activity (heart rate and blood pressure) that may contribute to fatigue, and neurophysiological measures of event-related potentials in OSA patients show slowing and amplitude changes consistent with sleep-deprived subjects. Thus several proposed mechanisms are likely to contribute to the cognitive dysfunction of OSA.

A meta-analysis of 28 studies examining neurobehavioural dysfunction in OSA found a comprehensive array of deficits over a similar range of domains as seen in the sleep deprivation literature. Specifically, moderate to large reductions were noted for performance on sustained attention tasks, simulated driving and working memory tasks. A more recent review by Jackson et al. reported that hallmark features of cognitive and daytime dysfunction in OSA are chronic excessive daytime sleepiness (measured objectively and subjectively), mood and cognition changes, and impaired vigilance and driving difficulties. In summary the cognitive deficits associated with OSA are similar to those of sleep deprivation, and likely contributed to by sleep fragmentation, sleep deprivation and intermittent hypoxaemia.

A real-world consequence of the chronic excessive daytime sleepiness and impaired vigilance associated with OSA is driving difficulties. The cognitive deficits identified in untreated OSA patients are reflected in patient report of difficulty concentrating, forgetfulness, poor decision making and falling asleep driving. These cognitive changes can have significant effects on daily living, and of great concern is the higher risk of motor vehicle crash and workplace accidents.
2.9 **DROWSY DRIVING**

Drowsy driving is an important contributor to motor vehicle crashes and road-accident fatalities, with sleepiness accounting for up to 20% of crashes on monotonous roads, especially highways.\textsuperscript{108} Driving is a complex task conducted by most people on a daily basis demanding perception, judgement and adequate response time. It is an applied task requiring attention and vigilance, both of which are impaired by insufficient or poor quality sleep. Thus, an important outcome of inadequate sleep is driver sleepiness and increased risk of accidents. Estimates of the proportion of crashes attributable to driver sleepiness vary tenfold from 1-3% in the US,\textsuperscript{235} to 20% in New Zealand\textsuperscript{236} and 30% in Australia.\textsuperscript{237}

In the US, self-reported drowsy driving is regularly surveyed and prevalence has ranged from 51% of respondents to a peak of 60% in the 2005 National Sleep Foundation poll.\textsuperscript{238} The statistics for falling asleep driving were also alarming, with 13% of respondents reporting an episode once per month.\textsuperscript{238} Those who reported snoring (suggestive of OSA) or usually sleeping ≤ 6 hours per day were more likely to report falling asleep while driving. Thus these trends of drowsy driving suggest that the public health burden of sleepiness-related injuries is likely increasing. Motor vehicle crashes (MVCs) are the fifth leading cause of death and injury in the US and inattentiveness, fatigue and sleepiness are now recognised as possible primary contributing factors.\textsuperscript{108}
2.9.1 Features of drowsy driving crashes

It remains a challenge to accurately ascribe driver sleepiness as the cause of a crash due to the lack of easily applied onsite measurement tools for sleepiness. However, there are distinct patterns to sleep-related crashes by type, age and time of day. Sleep-related crashes are more likely to occur mid-afternoon and at night, when drivers are sleepy. In addition, drowsy driving crashes often involve a single vehicle leaving the road, with no evidence of braking.\textsuperscript{239} Rear-end and head-on crashes are likely to be sleep-related.\textsuperscript{240} Finally, drowsy driving crashes are more likely to result in major injury or fatality since no attempt is made to brake.\textsuperscript{241} Identification of these features of drowsy driving crashes has contributed to the estimation that fatigue and sleepiness may account for up to 20\% of MVCs in the general population.\textsuperscript{236}

2.9.2 Occupational groups at risk of drowsy driving

There has been particular focus on commercial drivers since the prevalence, severity and public health impact of crashes involving commercial vehicles is high.\textsuperscript{59} Long-haul commercial drivers are especially vulnerable to drowsy driving due to short average sleep duration and a high prevalence of OSA among drivers.\textsuperscript{14,242} The prevalence of OSA among commercial drivers has drawn special attention because of the high cost and fatality rates of truck and bus crashes. In the US in 2009, there were 1,547,797 police-reported motor vehicle crashes with injury or fatality, of which 63,197 involved large trucks and buses. Of the single-vehicle heavy truck crashes, 18\% of involved drivers admitted to having fallen asleep behind the wheel, and investigators concluded that 50\% were fatigue-related.\textsuperscript{243} Heavy vehicles are overrepresented in road deaths, with 12\% of
fatalities involving workers in the trucking industry. In 2009, the cost of commercial motor vehicle crashes was estimated at US$7,200,000 for a fatal crash and US$331,000 for an injury crash. Thus both the human and economic cost of motor vehicle crashes is high and unfortunately increasing.

Over the past ten years, several studies have been conducted in commercial drivers to establish the prevalence of OSA. Commercial drivers are vulnerable to the development of OSA due to the sedentary nature of the work, irregular work shifts, monotonous long-hours of driving and poor access to healthy food on shift, all factors that contribute to obesity. In Australia, Howard et al. found that 60% of commercial motor vehicle drivers had sleep disordered breathing and 24% were excessively sleepy. Most studies have found a much higher prevalence of OSA than the general population (5%). A study of taxi drivers in NZ found increased risk of OSA in 18% of drivers. A similar study of truck drivers in Brazil reported a prevalence of risk of 11.5%. Further work in Australia with long-haul truck drivers demonstrated that 41% were likely to have OSA. Taken together most studies examining the presence of OSA in commercial drivers have reported prevalence in the range of two to six times that of the community. These data highlight the need to assess for OSA risk in all patients who drive professionally.

2.9.3 **Fitness to drive**

For most people, driving is an essential part of modern life. A survey of OSA patients attending sleep clinics in the UK found that 82% held a current driving licence, 62% drove a vehicle, and 22% drove for a living (or held a commercial motor vehicle licence [16%]). Thus an important role for sleep clinicians is assessment of driving risk. Several international guidelines help determine fitness
to drive in both private and commercial drivers, giving specific guidance regarding management of excessive sleepiness and OSA. High-risk drivers are defined as those with severe daytime sleepiness (an ESS score ≥ 16) and a history of motor vehicle crashes or near-misses caused by inattention or sleepiness.\textsuperscript{108}

Current Australian Fitness to Drive guidelines recommend that sleepy individuals should be advised to avoid or limit driving, and not drive at all if high risk.\textsuperscript{17} Sleepy drivers and those with suspected OSA should be referred to a sleep specialist for evaluation and treated. Note that there is no compelling evidence to restrict driving in OSA patients when there has not been a crash or equivalent event, since studies suggest up to two-thirds of drivers do not have a crash.\textsuperscript{250,251}

Since crashes, injuries and fatalities involving large trucks and buses are a significant problem in Australia and internationally, and OSA is prevalent among commercial motor vehicle drivers, screening for OSA has been promoted for this group.\textsuperscript{16,42,245,252} Since OSA is treatable and treatment reduces crash risk,\textsuperscript{21,253} the potential benefits of OSA screening in commercial motor vehicle drivers are great. Substantial effort over the past ten years has led to the development of a more consistent framework to manage OSA screening, testing and treatment of commercial motor vehicle drivers.\textsuperscript{254} In 2008 portable sleep monitoring was approved partially on the basis to provide better access to diagnostic testing.\textsuperscript{245} Although the guidelines in place are provided for commercial drivers, private drivers also have increased risk, so expeditious diagnosis and treatment of OSA is of equal importance in this group.\textsuperscript{108} The challenge for the sleep physician is the management of crash risk, since prediction of risk in an individual is imprecise. The most recent systematic review of crash risk and OSA was inconclusive.
regarding the characteristics that may predict crash risk, suggesting that BMI, AHI, hypoxaemia and excessive daytime sleepiness may contribute.\textsuperscript{16} The study of motor vehicle crashes is hampered by their low occurrence rates and contentious nature. Studying near-miss incidents has potential advantages in this regard.

### 2.9.4 Near-misses

Near-misses have a similar profile to accidents, and safety research has shown that study of near-misses may identify relevant risk factors.\textsuperscript{255} A safety ‘incident’ or near-miss is defined as an event that, under slightly different circumstances, could have been an accident. In many areas of industry and transport, systems of reporting near-misses are routine since it is understood that focus on these more frequent events is likely to improve outcomes.\textsuperscript{255}

A driving research challenge in making an OSA crash risk association is that commercial motor vehicle crashes in particular are statistically rare events.\textsuperscript{256} Over the past ten years, increased attention has been paid to sleepy near-miss driving crashes due to their likely relationship with actual motor vehicle crashes. Powell \textit{et al.} conducted a large prospective survey in the US on driving behaviours and reported a statistically significant dose-response between sleepy driver near-misses and actual accidents.\textsuperscript{257} Similarly, Philip \textit{et al.} reported that where the ESS was greater than 15 in French drivers, sleepy driving accidents were associated with an odds ratio of 5.0.\textsuperscript{258} Thus a sleepy near-miss may represent a dangerous precursor to a MVC.
Driving risk research in OSA patients has analysed near-misses and motor vehicle crashes before and after CPAP treatment to demonstrate reduced risk with treatment. A recent meta-analysis reported a sizeable protective effect of CPAP for motor vehicle crashes, near-misses and driving simulator incidents. This effect was given context by analysing risk differences and numbers needed to treat for both motor vehicle crashes and near-misses. The application of CPAP treatment was associated with an estimated 55% and 77% reduction in motor vehicle crashes and near-misses, respectively. The numbers needed to treat calculation was devised as a simple way to summarise the investment of time, energy and public health resources towards achieving a specific therapeutic goal. For example, an assessment of the impact of aspirin treatment to reduce heart attack and stroke found that numbers needed to treat was 73 for total heart attack, 278 for fatal heart attack and 256 for ischaemic stroke. By comparison, the substantially smaller number needed to treat of five and two OSA patients to prevent one crash and near-miss, respectively, shows the significant public health benefit of CPAP use for OSA patients.

A benefit of reporting both motor vehicle crashes and near-misses is that near-misses occur at a higher frequency, thus improving the possibility of measuring statistical difference for events that are rare. The data reported in the meta-analysis found near-misses occurred in 21 to 82% of subjects, whereas crashes occur at a much lower frequency or not at all.

In summary, use of CPAP treatment for OSA is highly effective in the reduction of crash risk. From a health economic perspective it is also a highly cost
effective treatment across a range of measures as discussed further in Section 2.10.3.

2.9.5 Risk factors for drowsy driving

Drowsy driving can be due to excessive sleepiness, sleep deprivation, circadian rhythm changes due to shift work, fatigue, sedating medications and consuming alcohol when tired. Of the several risk factors for drowsy driving, the greatest is likely to be sleep loss due to insufficient sleep or poor sleep hygiene. The scale of sleep loss in society has been investigated over the past thirty years by annual sleep health polls (since 1991) in the US and more recently in Australia. Evaluation of sleep habits has shown that frequent sleep difficulties, daytime fatigue, sleepiness and irritability are highly prevalent (20-35%) in society. Since drowsiness slows reaction time, causes inattention and impairs decision making, there is a clear mechanism for sleep loss to contribute to increased crash risk.

The most common contributors to fall-asleep crashes are working multiple jobs, night shift work and sleep duration of less than 5 hours, all of which are linked to insufficient sleep. In broad terms, the sleepiness contributing to drowsy driving can be caused by societal factors (such as work schedules, round-the-clock access to technology and family responsibilities), environmental influences (sedating medications and alcohol), sleep disorders, or a combination of these factors. Since OSA is the most common of the sleep disorders, the relationship to drowsy driving and motor vehicle crashes has gained increased attention as discussed in the following section.
2.9.6 Obstructive sleep apnoea and motor vehicle crashes

Obstructive sleep apnoea is the most common sleep disorder that causes excessive daytime sleepiness, and is thus a risk factor for both drowsy driving and fall-asleep MVCs. It has been heavily researched with respect to MVCs due to the high prevalence of OSA among professional drivers (ranging from 26 to 50%). In addition, many OSA patients report sleep-related crashes and near-misses to their clinicians, raising obvious concern.

Research in both clinical cohorts of OSA patients and community-based samples of drivers found that OSA is associated with a 2-3 times increased risk for motor vehicle crashes. A meta-analysis examining impairment and crash risk associated with ageing and disease found that OSA had the highest relative risk [RR: 3.71 (95% CI: 2.1, 6.40), p < 0.001] of all conditions considered. There have been two systematic reviews of OSA and driving risk, the most recent being commissioned in the US to assist with the development of guidelines and standards for commercial motor vehicle drivers. It was confirmed that the mean crash rate ratio associated with OSA is likely to fall in the range of 1.21 to 4.89, however prediction of risk in an individual is imprecise. Not all patients have crashes, and as many as two thirds may never have a collision. Thus identifying those at greatest risk remains a challenge.

The literature was reviewed with the specific intention of defining factors to enhance prediction of risk for occupational drivers, enabling clinicians to improve their ability to differentiate between low and high-risk individuals for fitness to drive. Sufficient data was available to evaluate the following
characteristics that may predict crash risk in drivers with OSA: obesity, disease severity (AHI), excessive daytime sleepiness and hypoxaemia.

2.9.7 OSA factors that may predict crash risk: Obesity

Obesity is a common risk factor for OSA, with 60 - 90% of subjects presenting with a BMI greater than 30 kg/m$^2$. Tregear et al. reported that higher BMI is a risk factor for motor vehicle crash in individuals with OSA, based upon strong evidence from four studies.$^{16}$

2.9.8 OSA factors that may predict crash risk: Hypoxaemia

The repetitive hypoxia associated with OSA has been implicated as a potential cause of both the symptom of excessive daytime sleepiness and the metabolic dysfunction implicated in the increased morbidity and mortality associated with OSA.$^{190}$ Although hypoxia is a physiological parameter that is routinely measured during sleep and frequently seen in OSA, no association was found between hypoxaemia and crash risk.$^{16}$ It should be noted however that the five studies examined for this association were rated as low quality, and thus this evidence does not rule out hypoxaemia as a potential risk factor.

2.9.9 OSA factors that may predict crash risk: Excessive daytime sleepiness

Fatigue or sleepiness is one of the major causes of motor vehicle crashes.$^{108}$ Since excessive sleepiness is a common symptom in OSA, it is a likely contributor to increased crash risk in these individuals. Most studies that have examined the relationship between excessive daytime sleepiness and crash risk in OSA populations have utilised the ESS score as the sleepiness measure. There was a trend towards an increased crash risk with higher ESS scores, but the finding was
not statistically significant \((p = 0.061)\).\textsuperscript{16} A recent study by Karimi \textit{et al.} found that severe daytime sleepiness \((\text{ESS} \geq 16)\) increased risk of crash in OSA patients \((\text{OR}: 2.13, 95\% \text{ CI: } 1.26, 3.61, p = 0.005)\).\textsuperscript{272} Two studies used the objective measure of sleep latency on MSLT, but neither found significant differences between OSA drivers with and without motor vehicle crashes.\textsuperscript{268,273} Thus there is not strong evidence for the two most widely used measures of excessive daytime sleepiness \((\text{ESS} \text{ and MSLT})\) to predict crash risk in individuals with OSA.

### 2.9.10 OSA factors that may predict crash risk: Disease severity

The data relating disease severity and crash risk among OSA populations is mixed, with three studies finding that severity of OSA was associated with increased risk of crash, while five others found no association.\textsuperscript{16} Tregear \textit{et al.} included three studies in the calculation of an effect-size estimate. Results suggested a trend toward greater severity of OSA \((\text{as measured by AHI})\) among those with OSA who crashed, but this was not statistically significant \((p = 0.055)\).\textsuperscript{274-276} Recently, Karimi \textit{et al.} reported that OSA severity \((\text{AHI})\) failed to identify patients at risk.\textsuperscript{272} Thus the intuitive idea that an individual with severe OSA has a higher risk of crash than one with mild OSA is not supported by current evidence.

In summary, the 2009 meta-analysis of studies examining drivers with OSA concluded that body mass index, AHI, hypoxaemia, and excessive daytime sleepiness may predict crash risk.\textsuperscript{16} However, the relative importance of these risk factors remains unclear since the conclusions were drawn from studies with small sample size, limited confounder adjustment and use of variable measurement technologies and scoring rules to define disease thresholds.
A recent well-designed study from Sweden has attempted to redress the limitations of past research, and extended the current literature by assessing the impact of CPAP treatment adherence. Their study confirmed an elevated risk of crash associated with OSA, but strengthened the evidence by using an objective measure of crash (police reports), assessed crash risk using both prospective and retrospective techniques and compared the OSA cohort to an external cohort. Disease severity (AHI) did not predict crash risk, but severe excessive daytime sleepiness (ESS ≥ 16), short habitual sleep time (≤ 5h/night) and use of hypnotics were associated with increased crash risk. An important novel finding was that CPAP use ≥ 4h/night was associated with a reduction of crash incidence, confirming the conclusions of the CPAP meta-analysis (Section 2.9.4). The results of the Karimi et al. study highlight the notion that individuals vary in their susceptibility to sleep loss, and future studies should focus on alternative objective markers of sleepiness or cognitive function to better identify those OSA patients most at risk (Section 2.8.5).

Driving is a complex task requiring alertness, vigilance, complex higher cortical function and motor skills. The most obvious cause for impaired driving in OSA patients is excessive daytime sleepiness, but the literature is inconsistent at this time. Recent data from neuroimaging techniques have shown significant changes to brain structure and metabolism in OSA patients. Thus the effects of neural, cognitive and daytime functional impairments, in addition to excessive daytime sleepiness, may contribute to increased risk of motor vehicle crashes in OSA (as discussed in Section 2.8.6).
2.10 The economic cost of undiagnosed OSA

There is growing concern that chronic sleep loss and sleep disorders have a substantial effect on an individual’s performance, safety and quality of life. At a societal level, disrupted sleep quality leads to cognitive deficits with significant downstream effects on daily function, including increased risk of driving and workplace accidents. Over the past ten years increased consideration has been given to the significant economic impact of sleep loss and sleep disorders on society. Both the direct and indirect financial costs of sleep disorders have been estimated, in addition to the non-financial costs derived from loss of quality of life and premature death. Such an approach provides the framework against which the nature and magnitude of these costs can be measured, to assess the cost benefit of treating the most prevalent sleep disorders such as OSA.

2.10.1 Estimation of the direct costs of OSA

Relatively little has been published on the economic implications of sleep disorders, but the direct medical costs of OSA have been examined due to the rising prevalence of the condition. Direct health costs include visits to health care professionals, diagnostic tests, treatment costs and hospital services for OSA itself and the medical conditions occurring as a result of OSA. Analysis of the direct health care costs of individuals with OSA has been made in both the US and Australia. The most recent estimates were made in Australia in 2011 when the Sleep Health Foundation commissioned Deloitte Access Economics to undertake an analysis of the direct and indirect costs associated with sleep disorders for 2010. Sufficient data was available to examine costs for the three most common sleep disorders – OSA, primary insomnia and restless legs syndrome. The total health
care costs associated with OSA were $657 million (AUD) for OSA itself and $409 million for the health costs of conditions attributable to OSA. The conditions attributable to OSA include motor vehicle crashes and workplace accidents, hypertension, vascular disease and depression.

In Canada and the US, similar attempts have been made to estimate direct costs and the burden of OSA on the health system. Kapur et al. found that in the year prior to diagnosis, the medical expenses of OSA cases were double that of controls. Given that about 80 to 90% of OSA cases remain undiagnosed and untreated, the burden of the disorder on the health system will be substantial. In 2006, Colten et al. took the figures for undiagnosed OSA and the cost of polysomnography and calculated that it would cost $17.5 billion to test and $3 billion to treat everyone in the US with OSA. Thus the total direct costs associated with testing and diagnosis of OSA alone is very high. It is recognised that until more effective portable sleep monitoring systems are introduced the burden of testing will remain high. Thus there is an imperative to validate simple home portable sleep monitors to expedite diagnosis and treatment of at-risk OSA cases.

2.10.2 Estimation of the indirect costs of OSA

The indirect costs of sleep disorders are broad and include those derived from illness-related morbidity and mortality borne by the patient and the employer in the form of workplace and driving accidents, absenteeism, loss of productivity, disability, hospitalisation and increased medical costs. In Australia in 2010, the indirect financial costs were much greater than the direct costs ($4.3 billion), and included $3.1 billion in lost productivity and $650 million in informal
care and other indirect costs from motor vehicle crashes and workplace accidents. Obstructive sleep apnoea (OSA) accounted for 61% of these indirect costs. If the non-financial costs estimating the effect of sleep disorders on loss of quality of life are considered, an additional $31.4 billion must be added, which represents six times the total financial cost.\textsuperscript{1}

Since drivers with OSA have a higher rate of motor vehicle crashes than control subjects, several analyses have been conducted to estimate the cost of crashes in both private and commercial drivers. An early analysis by Leger reported the costs of motor vehicle crashes attributed to sleepiness to be between US$29.2 and US$37.9 billion.\textsuperscript{105} More detailed analysis of fatal truck crashes found that over 50% were caused by sleep-related fatigue.\textsuperscript{54,260} The large expenses incurred by drowsy driving motor vehicle crashes prompted further research and cost analysis associated with crashes in OSA drivers. Do public health and clinical programs aimed at reducing sleepy driver incidents yield significant economic savings?\textsuperscript{267} In 2000 in the US, Sassani et al. conducted an analysis of OSA-related motor vehicle crashes and concluded that treating all OSA drivers with CPAP would cost US$3.2 billion, but would save US$11.1 billion and 980 lives annually.\textsuperscript{284} This work was taken a step further to tackle the issue of the potential cost-effectiveness of screening and treating commercial motor vehicle drivers.

In a group of commercial motor vehicle drivers, a comparison was made between diagnosis by PSG, screening then selective PSG for high-risk drivers or not screening at all.\textsuperscript{285} The most cost-effective option was found to be screening with BMI, age and gender, followed by confirmatory PSG on high-risk drivers. This group concluded that in the future, an initial screen followed by home portable
sleep study could result in substantial gains in cost-effectiveness.\textsuperscript{285} Taken as a whole, research into OSA-related motor vehicle crashes demonstrates a huge financial burden, and furthermore, CPAP treatment reduces comorbidities associated with OSA, incidence of motor vehicle crashes\textsuperscript{21} and healthcare costs and utilisation.\textsuperscript{286}

2.10.3 \textbf{Economic impact of OSA}

The costs of delivering medical care are high, and as such there is an imperative for practitioners and government to scrutinise costs and outcomes to ensure the best use of resources to address health without compromising safety.\textsuperscript{279} Attempts to estimate the economic cost of sleep disorders and specifically OSA have revealed that the healthcare costs and resource utilisation of undiagnosed OSA is enormous, costing billions of dollars per year.\textsuperscript{53,287,288}

The cost-effectiveness of CPAP therapy for OSA versus no treatment has been evaluated in many countries and found to be an effective use of healthcare resources.\textsuperscript{287} Albarrak \textit{et al.} analysed the health care usage of 342 OSA patients (with matched controls) on CPAP for five years both prior and after treatment started.\textsuperscript{286} The group concluded that treatment of OSA reversed the trend of increasing healthcare utilisation seen prior to diagnosis, and thus represented a long-term health benefit.\textsuperscript{286} The most recent assessment reported that CPAP is highly effective according to World Health Organisation guidelines and demonstrates a dominant effect from a societal perspective, meaning saving both money and healthy life.\textsuperscript{1} In other words, the costs of leaving the most prevalent sleep disorders untreated are far more than the costs incurred by delivering adequate treatment.\textsuperscript{1,59} To provide perspective relative to other chronic
conditions, the cost of sleep disorders, when extrapolated to the US population, would be greater than the economic burden of asthma and chronic obstructive pulmonary disease (COPD), and similar to that of diabetes.\textsuperscript{287} Thus sleep disorders contribute to a heavy public health burden.

2.10.4 \textbf{Sleep disorders and public health}

Estimation of the economic cost of sleep disorders to society has highlighted an enormous public health problem. Sleep loss, no matter the cause, has the potential to create serious consequences for both the individual and society. This is particularly true for OSA since most cases (80-90\%) remain undiagnosed and untreated, placing the healthcare system and society under pressure. Economic analysis has shown that the direct medical costs to diagnose and treat OSA alone are large. Motor vehicle crashes related to OSA represent an additional financial burden. Of even more concern are the huge indirect and non-financial costs which total billions of dollars, and are similar in scale to comparable chronic illnesses such as asthma and diabetes.

The sleep medicine community is aware of this burden, but effort is required to translate research advances into educational messages for the community and government policy makers, to improve public health and safety.\textsuperscript{54} The American Academy of Sleep Medicine has recently commissioned the Adult OSA Quality Measures Workgroup with the goal to optimise care for adult patients with OSA.\textsuperscript{288} The three primary outcomes selected are to improve disease detection and categorisation, improve quality of life and reduce cardiovascular risk. This approach is timely and has the opportunity for success since treatment can reduce
costs, and in many cases can improve quality of life and reduce morbidity from symptoms and comorbid conditions.

2.11 SUMMARY

There are three key areas of incomplete knowledge to be addressed by this thesis, each connected with the important symptom of daytime sleepiness and its relationship to OSA and accident risk:

1. One of the highest risks associated with untreated OSA is that of MVCs due to the frequently associated symptom of excessive daytime sleepiness. From a clinical perspective there is a need to understand which individuals with probable OSA are at increased risk of MVCs. Currently prediction of risk is imprecise. Current fitness to drive guidelines recommend not driving when a person is very sleepy (ESS > 16). Less sleepy individuals are also at increased risk. Do the symptoms of fatigue and impaired concentration confer increased crash risk? Legal levels of blood alcohol may have detrimental additive effects to excessive sleepiness. The economic burden associated with OSA-related MVCs is high both in Australia and internationally. Identification of OSA patients at greatest risk of MVCs or work-related accidents has the potential to reduce the public health burden of OSA.

2. There is great individual variability in the susceptibility to sleepiness in the context of sleep disorders or sleep restriction, which appears to be a stable individual trait. What are the components of an individual sleep phenotype or trait? Sleepiness, diurnal preference and sleep duration are recognised as heritable traits, but the genetic basis is largely unknown. Specific circadian
rhythm genes may play a role in the perception of tiredness or sleepiness, and this may influence crash risk in a sleep clinic population. The sleep disruption caused by OSA results in a sleep debt, and individuals may have different cognitive responses to OSA and sleep loss. It is possible that individuals with particular ‘susceptibility’ polymorphisms are more vulnerable to sleep debt, and identification of such individuals would provide the opportunity to develop improved risk models for the management of accident risk. Genetic polymorphisms predicting susceptibility to sleep loss have never been examined in individuals with OSA.

3. The burden of undiagnosed OSA in the community is high. The current gold standard investigation of polysomnography is costly and demand exceeds capacity. There is a need for validated cost-effective diagnostic tools to expedite triage of suspected OSA cases, especially those who are very sleepy and at risk of accidents. There is a paucity of well-designed validation studies of simple Type 4 portable monitors in particular, and a consequent reluctance for professional associations representing sleep medicine to endorse the use of portable sleep monitors for the diagnosis of OSA. With the focus on public safety, it is imperative that strategies are implemented to improve disease detection and categorisation. Clinical research studies are needed to optimise the use of portable sleep monitors in the clinical diagnostic pathways of sleep clinics and for community-based screening.
3 SELF-REPORTED MOTOR VEHICLE CRASHES AND OBSTRUCTIVE SLEEP APNOEA IN WA

3.1 Foreword

This chapter addresses the association between obstructive sleep apnoea (OSA) and the risk of motor vehicle crashes (MVCs) and near-misses. A primary symptom of OSA is excessive daytime sleepiness, which is known to contribute to increased risk of both occupational and driving accidents.\textsuperscript{37,59} It has been shown that subjects with OSA are more likely to have a MVC than those without sleep apnoea,\textsuperscript{5} but the factors predicting increased risk have not been clearly identified despite extensive research. This is of particular concern for commercial drivers who have high driving exposure and work night shifts where crash risk is greater.\textsuperscript{23,6,293} Sleep physicians have responsibility for assessing driving risk when a patient presents for OSA investigation, and clear evidence defining those aspects of OSA that confer most risk would aid management. Accordingly, the purpose of this study was: i) to describe the incidence rate of motor vehicle crashes in patients with OSA in our WASHS cohort, and ii) to investigate MVC risk factors in OSA patients. The research described in this chapter was published in the \textit{Journal of Clinical Sleep Medicine}.\textsuperscript{294}
3.2 ABSTRACT

**Study Objectives:** 1) To describe the incidence rate of motor vehicle crashes (MVCs) in patients with obstructive sleep apnoea (OSA); and 2) to investigate MVC risk factors in OSA patients.

**Methods:** A retrospective case-series observational study was conducted using data from the Western Australian Sleep Health Study at a tertiary hospital-based sleep clinic. Participants were patients (n = 2,673) referred for assessment of suspected sleep-disordered breathing. Questionnaire data were collected including age, sex, years of driving, near-misses and motor vehicle crashes, sleepiness and consumption of alcohol and caffeinated drinks. Overnight laboratory-based polysomnography was performed using standard methodology. Poisson univariate and negative binomial multivariable regression models were used to investigate associations between risk factors and MVC and near-miss risk in patients with untreated OSA.

**Results:** In patients with untreated OSA the crash rate was 0.06 MVC/person-year compared with the general community crash rate of 0.02 MVC/person-year. The rate ratio comparing very sleepy men with normal men was 4.68 (95% CI 3.07, 7.14) for near-misses and 1.27 (95% CI 1.00, 1.61) for crashes, after adjusting for confounders. In women there was a significant association with sleepiness score (p = 0.02) but no dose effect across quartiles.
Conclusions: Untreated OSA is associated with an increased risk of near-misses in men and women, and an increased risk of motor vehicle crashes in very sleepy men. There is a strong association between excessive daytime sleepiness and increased report of near-misses. Our data support the observation that it is those patients with increased sleepiness regardless of OSA severity that are most at risk.

3.3 Introduction

Obstructive sleep apnoea (OSA) is often associated with excessive daytime sleepiness and is a significant independent contributing factor to motor vehicle crashes (MVCs). Patients with OSA are up to seven times more likely to have a MVC than those without sleep apnoea, and driver sleepiness has been identified as one of the major causes of highway accidents and fatal crashes. It has been shown that personal injury from crashes is more common in OSA patients, and accidents involving OSA patients are more likely to be associated with major injury. This is probably due to the fall-asleep nature of these crashes.

Sleep physicians are regularly confronted with the management dilemma of diagnosing the pathology and treatment of sleepiness in occupational drivers. The prevalence of OSA among commercial drivers has been extensively investigated because crashes involving trucks and buses are particularly expensive, calamitous and associated with higher fatality rates. In a large study of Australian commercial vehicle drivers, Howard et al. found that 60% of drivers had sleep disordered breathing and 24% were excessively sleepy. In 2012 Sharwood reported that 41% of long-distance heavy vehicle drivers were likely to have sleep apnoea. A recent study conducted in the United Kingdom demonstrated high
rates of sleepiness and sleep-related accidents among bus drivers and the authors concluded that screening for OSA among this group should be seriously considered. However, there remains a need to establish thresholds of disease severity for sleep apnoea that guide driving restriction in a similar way as for levels of alcohol consumption.

It is unclear which risk factors associated with OSA contribute to motor vehicle crashes. The role of disease severity and daytime sleepiness has been assessed by systematic review. Risk of motor vehicle crashes in non-commercial drivers with OSA was associated with increased sleepiness in half of the studies reviewed. A 2009 meta-analysis of studies examining commercial drivers with OSA concluded that the disease-related factors of body mass index (BMI), apnoea hypopnoea index (AHI), hypoxaemia and daytime sleepiness may predict crash risk. However, these conclusions were drawn from studies that had small sample sizes (most < 200 subjects), limited adjustment for confounders, and used various measurement technologies and scoring rules to diagnose OSA thresholds. Thus the relative importance of these risk factors remains unclear.

Further problems with the study of accident events are their low occurrence rates and their contentious nature, which creates barriers to data collection. Studying near-miss incidents has advantages in these respects. It is well recognised in safety research that near-misses have a similar profile to accidents and that study of near-misses can identify factors that either exacerbate or mitigate risk. There has been little attention given to sleepy near-miss driving crashes in the sleep research literature despite their likely relationship with actual driving crashes. However, there have been two large studies of sleepy driver
near-misses that suggest such incidents may represent indicators of the presence of factors that increase risk of an actual accident.257, 37

The purpose of this study was to determine whether the risk of motor vehicle crashes was higher in patients with OSA than in the general community and, if so, the nature of the risk. To do so, we examined the relationships of near-misses and motor vehicle crashes with OSA severity, degree of daytime sleepiness and other potential risk factors in a sleep clinic population with a study design powered to address these associations using standardised measurement technologies for OSA.
3.4 METHODS

3.4.1 Study Sample

Patients referred to the Western Australian Sleep Disorders Research Institute (WASDRI) for investigation of sleep disorders from January 2006 to April 2009, who had questionnaire and sleep study data available, were included in the study. These patients were part of the West Australian Sleep Health Study (WASHS) cohort. Non-drivers were removed from the sample.

The WASHS is a resource established for clinical and genetic epidemiological investigation of sleep disorders and comprises subjects referred to WASDRI, the largest tertiary referral centre for sleep disorders in Western Australia. Of referred subjects, 98% gave consent to participate in the WASHS. The study was approved by the Human Research Ethics Committee at Sir Charles Gairdner Hospital (No. 2004-083).

3.4.2 Techniques

3.4.2.1 Questionnaires

Prior to clinical and PSG evaluation, a self-administered questionnaire was used to collect data on age, sex, years driving, motor vehicle crashes, near-misses, sleepiness (Epworth Sleepiness Score, ESS), and consumption of alcohol and caffeinated drinks.

Weekly alcohol consumption was calculated from the average number of alcoholic drinks (a standard [10 g alcohol] alcoholic drink) consumed in a typical week over the past year and level of consumption, which was based on their
response to the question “On days when you drink alcohol, how many drinks of beer, wine and/or other type of alcohol would you have?”. Response options were: 0, 1-2, 3-5, 6-9 or 10 or more. Caffeine intake was based upon the response to the question “During the past month, have you used coffee, tea or other caffeine drinks to stay awake during your normal waking time”? Possible responses were: never, rarely (has occurred but less than once a week), sometimes (1-2 times per week), frequently (3-4 times per week), always or almost always (5-7 times per week), or don’t know.

MVC data represent all self-reported near-misses and crashes for an individual driving history. To assess driving behaviour, subjects answered a series of six questions about ever having driven, years of driving, falling asleep whilst behind the wheel, near-misses and crashes. For near-misses, the question asked was “How many ‘near-miss’ car accidents have you had due to sleepiness?“ For crashes, the question asked was “How many car accidents have you ever had while driving a car?”

3.4.2.2 Polysomnography (PSG)

Overnight laboratory-based polysomnomography was performed using the Compumedics E-Series (PSG Online 2, Compumedics Ltd, Abbotsford, Australia) and analysed according to recommendations published by the American Academy of Sleep Medicine. Sleep was documented using standard electroencephalographic, electro-oculographic and electromyographic criteria. The PSG methodology for WASHS subjects is described in detail in the cohort profile paper.
Other measurements included electrocardiogram, nasal flow (measured using a nasal cannula/pressure transducer system), oronasal airflow (thermocouple) and thoracic and abdominal plethysmography. Oxygen saturation was measured by pulse oximetry using a finger probe (Nonin XPod 3012 with a Nonin 7000A finger probe [sample rate 1 Hz]; Nonin, Hudiksvall, Sweden). Periodic limb movements were recorded using piezo electric sensors positioned over the anterior tibialis muscle of each lower limb.

Obstructive apnoeas were defined as a decrease in airflow by 80% of baseline (duration 10-80 s) associated with ongoing respiratory effort. Central apnoeas were defined as a decrease in airflow by 80% of baseline (duration 10-80 s) associated with no respiratory effort. Obstructive hypopnoeas were defined as a ≥ 50% decrease in airflow (nasal pressure) or a clear but lesser decrease in airflow if associated with either a 3% desaturation or an arousal in the context of ongoing respiratory effort. The complete PSG record was reviewed manually by trained sleep scientists for sleep stage, leg movements, arousals, and respiratory events according to the recommendations published by the AASM using Profusion 2 software (Compumedics Ltd, Abbotsford, Australia).85 AHI and the arousal index (ARI) were defined as the number of events per hour of sleep.

3.4.2.3 Data analysis - general

Data were analysed using IBM SPSS Statistics (GradPack version 17.0 Release 17.0.2, March 11, 2009)298 and R (Version 2.14.1, 2011-12-22) and statistical significance was set at 5%. All data are reported as mean ± standard deviation (SD) or, if skewed, median [interquartile range (IQR)]. Male and female subject
characteristics were compared using independent t-tests or Mann-Whitney U tests. The analysis process is described diagrammatically in Figure 3.1.

Subjects were stratified by gender, then divided into four groups of OSA severity based upon their AHI, and into four quartiles of subjective sleepiness according to their ESS (Table 3.1).

<table>
<thead>
<tr>
<th>AHI (events/h)</th>
<th>OSA severity</th>
<th>ESS Quartile</th>
<th>Sleepiness Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to ≤ 5</td>
<td>Normal</td>
<td>0 to 5</td>
<td>Normal</td>
</tr>
<tr>
<td>5 to ≤ 15</td>
<td>Mild</td>
<td>6 to 9</td>
<td></td>
</tr>
<tr>
<td>15 to ≤ 30</td>
<td>Moderate</td>
<td>10 to 14</td>
<td>Excessive sleepiness</td>
</tr>
<tr>
<td>≥ 30</td>
<td>Severe</td>
<td>15 to 24</td>
<td></td>
</tr>
</tbody>
</table>

OSA, obstructive sleep apnoea; ESS, Epworth sleepiness score; AHI, apnoea hypopnoea index.

3.4.2.4 Data analysis – Driving

Near-miss and MVC count data from the questionnaire were converted to rates by dividing by years driving to allow comparison with community data. The overall crash rate for subjects with OSA (AHI > 5 events per hour) was compared with the crash rate for the general community. Near-misses were categorised for all subjects and the proportion of subjects with at least one crash calculated, in addition to the proportion of subjects with near-misses who fell asleep driving. Linear-by-linear association tests were used to assess relationships between these variables.
The proportion of missing data for crash questions was calculated to assess reporting bias. Chi-square tests were conducted across ESS quartiles (Quartile 1 normal as reference category) for each driving question to assess differences in the proportion of missing data relative to sleepiness.

3.4.2.5 Analysis of aggregated data: Poisson regression

Initial analysis was of aggregated data: the near-misses, crashes and years driving were aggregated for patients by ESS quartiles and OSA categories (Figure 3.1). To compare near-misses and crashes associated with severity of OSA (AHI category) and excessive daytime sleepiness (ESS quartile), univariable Poisson regression models (with offset log years driving) were used to estimate rate ratios (RRs) and 95% confidence intervals (CIs). Each category was compared to the normal (lowest) category. Trends across categories were assessed with Cochrane Armitage trend tests. Gender differences within each category of AHI and ESS were compared using rate ratio tests.
Crash Analysis

Case series N=2673
- Men (M) = 1696
- Women (W) = 977

OSA severity, categorised by AHI:
- Nil (M=65, W=115)
- Mild (M=295, W=289)
- Moderate (M=454, W=287)
- Severe (M=882, W=286)

Quartiles of sleepiness, categorised by ESS:
- 0-5  (M=418, W=248)
- 6-9  (M=433, W=232)
- 10-14  (M=465, W=289)
- 15-24  (M=378, W=204)

Analysis of aggregated data
Near-miss and MVC rates compared across OSA and ESS categories:
- Univariable Poisson regression models, with offset log years driving
- Gender differences analysed within each category of AHI and ESS using RR tests

Analysis of individual data
- Negative binomial regression with log link, offset log years driving
- Single variables, squares and interaction terms tested for significance
- Multivariable models developed for near-misses and MVCs, incorporating adjustment for potential confounders
- Parsimonious models developed

Figure 3.1 Flow diagram illustrating the analysis process

Footnote: MVC, motor vehicle crash; AHI, apnoea hypopnoea index; ESS, Epworth Sleepiness Score; OSA, obstructive sleep apnoea; RR, rate ratio
3.4.2.6 Analysis of individual data: Negative binomial regression

Subsequent analyses of individual data used negative binomial models (negative binomial regression with log link and offset log years driving) to test associations between each of the primary risk factors with near-miss and MVC rates (Table 3.2). Models were adjusted for potential confounding variables (body mass index (BMI), neck circumference (NC), proportion of time spent at an arterial oxygen saturation of < 90%, age, sex and alcohol and caffeine intake). Primary exposure and adjustment variables were included if significantly associated at the 20% significance level with each of the dependent variables. A summary of variables tested in models is given in Table 3.2. Full details of the multivariable regression analysis process are given in the Appendix 3.7, Multivariable regression analysis 3.7.1.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Near-misses or motor vehicle crashes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary exposure variables</strong></td>
<td>AHI (continuous and categorical), ESS (continuous, binary and quartiles) and ARI continuous</td>
</tr>
<tr>
<td><strong>Adjustment variables</strong></td>
<td>Age, NC, AHI or ESS score, years driving, proportion of time spent &lt; 90% SaO₂, alcohol and caffeine use</td>
</tr>
</tbody>
</table>

AHI, apnoea hypopnoea index; ESS, Epworth sleepiness score; ARI, arousal index; NC, neck circumference; SaO₂, arterial oxygen saturation.

Squares of continuous variables and interactions between variables were tested. As there was a significant interaction between each primary risk factor and sex, the dataset was stratified by gender to facilitate interpretation. Parsimonious models were developed by removing the least significant variable in a stepwise process.
until the only remaining variables had coefficients that were significantly different from zero. A parsimonious model is considered the simplest plausible model, and it is not a significantly worse fit than the full model. Each excluded variable was then re-tested one at a time in the parsimonious model.

3.5 RESULTS

3.5.1 Subject Characteristics

Questionnaire and sleep study data were available for 2,673 drivers. Table 3.3 presents study population characteristics by gender. Subjects were predominantly male, middle-aged and obese (BMI > 30), mean ± SD duration of driving was 30.2 ± 13.3 years, and ESS was 9.9 ± 5.5, which falls on the border of normal (range 0-9) and excessive sleepiness (range 10-24). For men and women combined, 17% reported moderate to severe excessive sleepiness (ESS ≥ 16). Age and ESS did not differ significantly between men and women, but men had significantly higher median scores than women for AHI (31 vs 18 events/h respectively; p < 0.001) and ARI (35 vs 28 events/h; p < 0.001), as well as lower BMI, lower minimum oxygen saturation, and higher proportion of time spent with an oxygen saturation < 90%, (Table 3.3).
Table 3.3. Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Men (N=1696)</th>
<th>Women (N=977)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50.0 ± 13.6</td>
<td>50.0 ± 13.5</td>
<td>0.99</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.8 ± 6.7</td>
<td>33.4 ± 9.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neck circumference, cm</td>
<td>43.3 ± 3.9</td>
<td>37.9 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lowest SaO₂</td>
<td>82.2 ± 11.0</td>
<td>85.5 ± 8.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time &lt; 90% SaO₂, min</td>
<td>1.7 (0.1-16.8)</td>
<td>0.2 (0-4.3)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Years driving</td>
<td>31.5 ± 13.5</td>
<td>28.1 ± 12.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESS, score</td>
<td>9.9 ± 5.4</td>
<td>9.8 ± 5.6</td>
<td>0.88</td>
</tr>
<tr>
<td>AHI, events/h</td>
<td>31 (17-56)</td>
<td>18 (9-34)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ARI, arousals/h</td>
<td>35 (24-52)</td>
<td>28 (19-40)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD or median (interquartile range); BMI, body mass index; SaO₂, arterial oxygen saturation; ESS, Epworth Sleepiness Scale; AHI, apnoea hypopnoea index; ARI, arousal index; *P value for independent t-tests.

3.5.2 Motor vehicle crashes and near-misses

At least one crash was reported by 69% of subjects. For 11% of the reported crashes, the driver had reported having a crash because they felt sleepy or fell asleep behind the wheel. A quarter of subjects (26%) reported at least one near-miss due to sleepiness and 32% reported having fallen asleep behind the wheel. Table 3.4 contains data on the association between the number of near-misses and both the percentage of subjects with at least one actual crash, and those who fell asleep at least once while driving. The number of near-misses has a linear relationship (p < 0.001) with the number of crashes and with fall-asleep driving episodes (Table 3.4).
Table 3.4 Relationship between near-misses and both crashes and episodes of falling asleep driving

<table>
<thead>
<tr>
<th>Number of near-misses due to sleepiness</th>
<th>Number of subjects</th>
<th>% of subjects with at least one crash</th>
<th>% of subjects who fell asleep driving at least once</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1910</td>
<td>66.1</td>
<td>19.8</td>
</tr>
<tr>
<td>1</td>
<td>210</td>
<td>68.6</td>
<td>69.7</td>
</tr>
<tr>
<td>2-3</td>
<td>241</td>
<td>76.3</td>
<td>63.5</td>
</tr>
<tr>
<td>4 or more</td>
<td>211</td>
<td>79.1</td>
<td>67.0</td>
</tr>
<tr>
<td>p value *</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

*p Chi-square linear-by-linear association

The mean overall crash rate of 0.06 MVC/person-year was three times higher (p < 0.0001) than the community crash rate of 0.02 MVC/person-year. The mean crash rate ratio for subjects with any OSA (AHI > 5 events per hour, n=2,486) versus those with no OSA (community) was 3.07 (95% CI 2.98-3.17). The proportion of missing data for crash questions ranged from 0.4 to 3.4%. There were no significant differences between ESS quartiles (quartiles 2, 3 and 4 compared with normal) for missing data (Pearson Chi-square p = 0.6 to 0.9).

3.5.3 Aggregated analysis: Univariable Poisson regression

3.5.3.1 Near-misses: Association with OSA category

In men there was a significant upward trend (p < 0.001) across OSA category (Table 3.5, Figure 3.2). As OSA severity increased, the number of near-misses reported increased by a factor of 1.1. Men with severe OSA reported near-misses 1.5 times more often than men with nil OSA (p = 0.01). For women, the opposite trend was seen (p < 0.001). As OSA severity increased, the number of near-misses
reported decreased by a factor of 0.82 (Figure 3.2). The RR for reported near-misses in women with severe OSA was 0.65, (95% CI 0.52-0.82). Thus, relative to women with nil OSA, those with severe OSA were 35% less likely to report near-misses (Table 3.5).

With respect to gender differences within AHI category, there was a significant difference between men and women in the normal, mild and severe categories (Figure 3.2 and Table 3.5).

![Figure 3.2. Near-miss rate and severity of OSA for men and women. Error bars represent 95% confidence intervals. Asterisks represent a significant difference between men and women (p < 0.001).](image)
Table 3.5. Rate ratios across OSA categories and ESS quartiles for near-misses in men and women.

<table>
<thead>
<tr>
<th>Near-misses</th>
<th>Men (N = 1696)</th>
<th>Women (N = 977)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>RR (95% CI)(^b)</td>
</tr>
<tr>
<td>OSA category</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>60</td>
<td>1.00</td>
</tr>
<tr>
<td>Mild</td>
<td>286</td>
<td>1.26 (0.80, 2.23)</td>
</tr>
<tr>
<td>Moderate</td>
<td>442</td>
<td>1.41 (0.72, 2.05)*</td>
</tr>
<tr>
<td>Severe</td>
<td>859</td>
<td>1.50 (0.68, 1.97)*</td>
</tr>
<tr>
<td>ESS quartile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>405</td>
<td>1.00</td>
</tr>
<tr>
<td>Non-sleepy</td>
<td>426</td>
<td>1.45 (1.21, 1.75)**</td>
</tr>
<tr>
<td>Sleepy</td>
<td>451</td>
<td>3.16 (2.69, 3.71)**</td>
</tr>
<tr>
<td>Very sleepy</td>
<td>363</td>
<td>6.13 (5.25, 7.15)**</td>
</tr>
</tbody>
</table>

ESS, Epworth Sleepiness Scale, OSA, obstructive sleep apnoea, \(^a\)Cochrane Armitage Trend Test, \(^b\)Poisson univariate regression, \(^*\)p<0.05, \(^**\)p<0.001, RR, rate ratio.

3.5.3.2 Near-misses: Association with subjective sleepiness

Comparison of near-miss rates across ESS quartiles found a statistically significant (p < 0.001) difference between rates across sleepiness categories in men and women (Table 3.5). Figure 3.3 illustrates a clear trend of increasing report of near-misses as ESS increased. Near-miss rate increased by a factor of 1.38 for women and 1.91 for men per quartile. With respect to gender differences within ESS quartile, men reported more near-misses than women in the moderate (p < 0.001) and severe sleepiness categories (p < 0.001), while women reported more near-misses than men in the normal (p < 0.001) and mild (p = 0.06) sleepiness categories (Figure 3.3).
Figure 3.3 Near-miss rate and subjective sleepiness for men and women. Error bars represent 95% confidence intervals. Asterisks represent a significant difference between men and women (p<0.001).

ESS, Epworth Sleepiness Scale.

3.5.3.3 Crashes: Association with OSA severity

Trend analysis showed a significant decrease (p < 0.001) in the risk of crashes across OSA categories in men (Table 3.6). For women there was no significant trend across categories (p = 0.98). However, relative to the nil OSA group, women with mild, moderate and severe OSA all reported less crashes with rate ratios of 0.65, 0.69 and 0.81 respectively (Table 3.6). With respect to gender differences within OSA category, men with mild and moderate OSA reported significantly (p < 0.001) more crashes than women (Figure 3.4).
### Table 3.6. Rate ratios across OSA categories and ESS quartiles for crashes in men and women

<table>
<thead>
<tr>
<th></th>
<th>Men (N = 1696)</th>
<th></th>
<th>Women (N = 977)</th>
<th></th>
</tr>
</thead>
</table>
|                      | n   | RR (95% CI)
|                      | Trend Test | n   | RR (95% CI) | Trend Test |
| OSA category         |     |        |                 |          |
| Normal               | 64  | 1.00   | <0.001          | 113      | 1.00  | 0.98 |
| Mild                 | 293 | 1.09 (0.89, 1.32) | <0.001          | 283      | 0.65 (0.54, 0.79)*** |
| Moderate             | 445 | 1.02 (0.84, 1.23) | <0.001          | 279      | 0.69 (0.57, 0.82)** |
| Severe               | 864 | 0.90 (0.75, 1.09) | <0.001          | 284      | 0.81 (0.68, 0.96)* |
| ESS quartile         |     |        |                 |          |
| Normal               | 412 | 1.00   | <0.001          | 241      | 1.00  | 0.40 |
| Non-sleepy           | 429 | 1.11 (1.01, 1.22)* | <0.001          | 226      | 1.40 (1.21, 1.63)** |
| Sleepy               | 452 | 1.11 (1.01, 1.22)* | <0.001          | 287      | 1.12 (0.97, 1.30)  |
| Very sleepy          | 371 | 1.23 (1.11, 1.36)** | <0.001          | 201      | 1.15 (0.98, 1.35)  |

ESS, Epworth Sleepiness Scale; OSA; obstructive sleep apnoea; *Cochrane Armitage Trend Test; †Poisson univariate regression; *p < 0.05; **p < 0.001; RR, rate ratio.
Figure 3.4. Crash rate and severity of OSA for men and women. Error bars represent 95% confidence intervals. Asterisks represent a significant difference between men and women (p < 0.001).

OSA, Obstructive sleep apnoea

3.5.3.4 Crashes: Association with subjective sleepiness

In men, there was a significant (p < 0.001) upward trend in crash rate with sleepiness (Table 3.6), whereas for women, there was no significant trend (p = 0.40). With respect to gender differences within quartile, men reported more crashes than women (p < 0.001) in the normal, sleepy and very sleepy quartiles (Figure 3.5).
Figure 3.5. Crash rate and subjective sleepiness for men and women. Error bars represent 95% confidence intervals. Asterisks represent a significant difference between men and women (p < 0.001).

ESS, Epworth Sleepiness Scale

3.5.4 Multivariable regression analysis

The models run for the continuous outcome variables of arousal index (ARI) or AHI found no associations for either near-misses or crashes at univariable or multivariable level, and were not examined further. Similarly, no relationship was found between OSA severity (as measured by AHI category) and either near-misses or crashes. There was a consistent relationship between ESS and increased risk of near-misses in men but not women, as shown in the unadjusted analyses (Table 3.5). The final multivariable models for near-misses and crashes, stratified by gender, are presented below.
3.5.4.1 Near-misses

Dichotomising the ESS variable failed to find a relationship across categories for women shown by the Poisson regression analysis (Table 3.5). Further models were run with ESS quartiles as the primary risk factor to test this association. The interaction between ESS quartile and sex was significant (p = 0.01), and thus data were stratified by gender.

The models estimating the relationship between ESS quartiles and near-misses (Table 3.7) showed a significant trend effect for men (p < 0.001) and women (p = 0.05). Men who were sleepy (ESS 10 to 14) or very sleepy (ESS 15 to 24) reported near-misses 2.9 and 4.7 times more often than those who were not sleepy (ESS 0 to 5). There was a significant trend across quartiles in women (p = 0.05), but no dose effect. As age increased subjects reported fewer near-misses (p < 0.001). With increasing caffeine use there was an increased probability of reporting a near-miss in men. Men and women reported near-misses 1.7 and 2.0 times more often with sometimes use of caffeine, and 2.3 and 2.6 times more often with frequent use of caffeine, respectively (Table 3.7).
### Table 3.7. Negative binomial regressions modelling near-misses, stratified by gender

<table>
<thead>
<tr>
<th>Near-misses</th>
<th>Men RR (95% CI)</th>
<th>p value</th>
<th>Women RR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESS quartile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Normal</td>
<td>1.00</td>
<td>&lt;0.001</td>
<td>1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>• Non-sleepy</td>
<td>1.02 (0.67, 1.55)</td>
<td>0.90</td>
<td>0.75 (0.32, 1.76)</td>
<td>0.51</td>
</tr>
<tr>
<td>• Sleepy</td>
<td>2.86 (1.86, 4.40)</td>
<td>&lt;0.001</td>
<td>0.92 (0.42, 2.01)</td>
<td>0.83</td>
</tr>
<tr>
<td>• Very sleepy</td>
<td>4.70 (3.07, 7.19)</td>
<td>&lt;0.001</td>
<td>1.69 (0.77, 3.72)</td>
<td>0.20</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.96 (0.95, 0.97)</td>
<td>&lt;0.001</td>
<td>0.93 (0.91, 0.95)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NC, cm</td>
<td>0.98 (0.94, 1.03)</td>
<td>0.26</td>
<td>1.03 (0.96, 1.10)</td>
<td>0.48</td>
</tr>
<tr>
<td>AHI</td>
<td>1.00 (0.99, 1.01)</td>
<td>0.72</td>
<td>1.00 (0.99, 1.01)</td>
<td>0.89</td>
</tr>
<tr>
<td>Time&lt;90% SaO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.98 (0.42, 2.25)</td>
<td>0.92</td>
<td>0.44 (0.10, 1.94)</td>
<td>0.28</td>
</tr>
<tr>
<td>Alcohol#</td>
<td>1.01 (1.00, 1.02)</td>
<td>0.19</td>
<td>0.98 (0.96, 1.01)</td>
<td>0.18</td>
</tr>
<tr>
<td>Caffeine use</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>• Nil</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Sometimes</td>
<td>1.72 (1.18, 2.52)</td>
<td>0.02</td>
<td>2.01 (1.14, 3.53)</td>
<td>0.02</td>
</tr>
<tr>
<td>• Frequent</td>
<td>2.27 (1.62, 3.18)</td>
<td>&lt;0.001</td>
<td>2.67 (1.62, 4.40)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

| Parsimonious model | | | |
|-------------------|-----------------|---------|-----------------|---------|
| ESS quartile      |                | <0.001  |                  | 0.02    |
| • Normal          | 1.00            |          | 1.00             |         |
| • Non-sleepy      | 1.01 (0.67, 1.53) | 0.96    | 0.76 (0.30, 1.94) | 0.56    |
| • Sleepy          | 2.87 (1.87, 4.42) | <0.001  | 0.96 (0.42, 2.17) | 0.91    |
| • Very sleepy     | 4.68 (3.07, 7.14) | <0.001  | 1.74 (0.75, 4.01) | 0.20    |
| Age, y            | 0.96 (0.95, 0.97) | <0.001  | 0.93 (0.91, 0.95) | <0.001  |
| Caffeine          |                | <0.001  |                  | <0.001  |
| • Nil             | 1.00            |          | 1.00             |         |
| • Sometimes       | 1.70 (1.16, 2.48) | 0.01    | 1.97 (1.12, 3.46) | 0.02    |
| • Frequent        | 2.27 (1.61, 3.18) | <0.001  | 2.64 (0.59, 4.40) | <0.001  |

ESS, Epworth Sleepiness Scale; NC, neck circumference; AHI, apnoea hypopnoea index; SaO<sub>2</sub>, arterial oxygen saturation; #alcohol, standard drinks per week; AHI*, AHI scaled by 10; RR, rate ratio; CI, confidence interval
3.5.4.2 Summary of analyses for near-misses

Multivariable negative binomial regression analysis showed that near-misses were associated with sleepiness, age and caffeine intake, but not with NC, AHI, oxygen saturation or alcohol intake (Table 3.7). After adjustment for significant confounders, very sleepy men were 4.68 times (95% CI 3.07, 7.14) more likely to report a near-miss than normal men (Table 3.7, parsimonious model). For women there was a significant trend across sleepiness quartiles (p = 0.05) but no dose effect. When this model was refitted with the very sleepy quartile as the reference category for ESS, the rates in the women’s non-sleepy and sleepy quartiles were significantly lower [Sleepy: RR 0.55, 95% CI 0.35, 0.86, p = 0.01; Non-sleepy: RR 0.44, 95% CI 0.23, 0.84, p = 0.01]. Caffeine use was significantly related (p < 0.001) to near-misses for both men and women and there was a dose effect (Table 3.7). In multivariable regression analysis with AHI considered as the primary categorical risk factor (and ESS as an adjustment continuous variable), AHI showed no significant effect for men (p = 0.71) or women (p = 0.80).

3.5.4.3 Crashes

Since a strong relationship between ESS and near-misses was demonstrated, a similar association may exist for crashes. The unadjusted analysis (Table 3.6) and histogram (Figure 3.5) illustrated the relationship of a general trend upward across ESS quartiles in men but not women. Negative binomial regression analysis was conducted on the gender stratified sample with the primary risk factor of ESS quartiles to investigate whether there was an association with crashes.
The final model showed no significant trend across sleepiness quartiles with crashes for either men (p = 0.18) or women (p = 0.46, Table 3.8). However, very sleepy men (ESS 15 to 24) reported crashes 1.3 times more often than non-sleepy men (ESS 0 to 5, p = 0.049). This relationship reflects the trend shown in Figure 3.5 (unadjusted analysis), but is not as strong as the relationship found between sleepiness and near-misses in men.

3.5.4.4 Summary of analyses for crashes

Multivariable negative binomial regression analysis showed that crash rate was related to age only and not sleepiness, AHI, NC, oxygen saturation, alcohol or caffeine intake in the full model (Table 3.8). In the parsimonious model, although very sleepy men reported crashes 1.3 times more often than normal men (p = 0.049), ESS did not have a significant association with crash rate.
Table 3.8  Negative binomial regressions modelling crashes, stratified by gender

<table>
<thead>
<tr>
<th></th>
<th>Crashes</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>RR (95% CI)</td>
<td>p value</td>
<td>Women</td>
<td>RR (95% CI)</td>
<td>p value</td>
</tr>
<tr>
<td><strong>Full model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESS quartile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.00</td>
<td></td>
<td>0.33</td>
<td>1.00</td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>Non-sleepy</td>
<td>1.09 (0.93, 1.27)</td>
<td>0.29</td>
<td>1.35 (0.90, 2.03)</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleepy</td>
<td>1.15 (0.95, 1.38)</td>
<td>0.15</td>
<td>1.03 (0.83, 1.29)</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very sleepy</td>
<td>1.22 (0.95, 1.56)</td>
<td>0.12</td>
<td>1.06 (0.83, 1.35)</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>0.97 (0.97, 0.98)</td>
<td>&lt;0.001</td>
<td>0.98 (0.97, 0.98)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC, cm</td>
<td>1.00 (0.98, 1.02)</td>
<td>0.80</td>
<td>1.02 (1.00, 1.04)</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHI</td>
<td>1.00 (0.99, 1.00)</td>
<td>0.10</td>
<td>1.00 (1.00, 1.00)</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time&lt;90% SaO\textsubscript{2}</td>
<td>0.79 (0.52, 1.19)</td>
<td>0.26</td>
<td>0.90 (0.46, 1.76)</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol#</td>
<td>1.00 (1.00, 1.01)</td>
<td>0.37</td>
<td>1.00 (0.99, 1.01)</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine use</td>
<td></td>
<td></td>
<td>0.09</td>
<td>0.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td>1.26 (0.96, 1.64)</td>
<td>0.10</td>
<td>0.98 (0.76, 1.25)</td>
<td>0.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequent</td>
<td>1.18 (0.97, 1.43)</td>
<td>0.10</td>
<td>1.05 (0.86, 1.29)</td>
<td>0.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Parsimonious model</strong></td>
<td></td>
<td></td>
<td>0.18</td>
<td>0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESS quartile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.00</td>
<td></td>
<td>0.18</td>
<td>1.00</td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>Non-sleepy</td>
<td>1.11 (0.95, 1.30)</td>
<td>0.19</td>
<td>1.38 (0.90, 2.09)</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleepy</td>
<td>1.18 (0.97, 1.43)</td>
<td>0.11</td>
<td>1.05 (0.84, 1.29)</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very sleepy</td>
<td>1.27 (1.00, 1.61)</td>
<td>0.049</td>
<td>1.11 (0.88, 1.40)</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>0.97 (0.97, 0.98)</td>
<td>&lt;0.001</td>
<td>0.97 (0.97, 0.98)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHI*</td>
<td>0.969 (0.948, 0.990)</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ESS, Epworth Sleepiness Scale; NC, neck circumference; AHI, apnoea hypopnoea index; SaO\textsubscript{2}, arterial oxygen saturation; #alcohol, standard drinks per week; AHI*, AHI scaled by 10; RR, rate ratio; CI, confidence interval
3.6 Discussion

This large study investigated risk factors for motor vehicle crashes and near-misses in sleep clinic patients. The study found that patients with untreated OSA (AHI > 5 events per hour) report crashes at a rate three times higher than the general community. However, in multivariable analysis the rates of reported crashes in males and reported near-misses in men and women were related more strongly to daytime sleepiness and not the severity of OSA. In patients with moderate to severe OSA or sleepiness, women tended to report fewer near-misses and crashes than men.

The mean crash rate ratio (3.07) for our patients with OSA is consistent with a recent meta-analysis which found that OSA was associated with a 1.21 to 4.89 increase in the mean crash rate ratio. The reported characteristics that predicted crashes in drivers with OSA included AHI, hypoxaemia during sleep, BMI, and possibly daytime sleepiness.16

This study found a strong association between sleepiness and increased rate of reported near-misses. There was also evidence of a relationship between increased sleepiness and crashes for men but not women. Very sleepy men (ESS 15-24) reported near-misses 4.68 (95% CI 3.07-7.14) times more often than normal men (ESS 0-5), (Table 3.7). For women there was a trend effect across quartiles (p = 0.02, Figure 3.3). When the parsimonious model was refitted with quartile 4 (very sleepy) as the reference category for ESS, the rates in quartiles 2 and 3 were significantly lower [Q2: RR 0.44, 95% CI 0.23, 0.84, p = 0.01; Q3: RR 0.55, 95% CI 0.35, 0.86, p = 0.01]. The relationship between sleepiness and increased risk of crash or near-miss has not been consistently demonstrated in past studies.16 Our results agree with one other study that
was adequately powered (n = 150) and adjusted for confounders, which found a significantly higher risk of near-misses in subjects with higher ESS scores.\textsuperscript{301}

More recently, two large community studies provided evidence that sleepy near-misses may be dangerous precursors to an actual accident and that ESS score had an independent association with having a near-miss or actual accident.\textsuperscript{37,257} We found a relationship between the number of near-misses and the number of actual crashes (p < 0.001). Disturbingly, there was a relationship of similar magnitude between near-misses and fall-asleep driving episodes. Thus our study confirms the findings of Powell and Philip who both reported a relationship between sleepiness at the wheel, near-misses and actual crashes in large community-based samples.\textsuperscript{257,258}

Patients who are referred to sleep clinics show higher levels of daytime sleepiness than the general population.\textsuperscript{175} The mean ESS of 9.9 for our patients was approximately double the mean ESS of 4.6 seen in 72 subjects without sleep disorders,\textsuperscript{300} and the data show that sleep clinic patients with an ESS >10 have approximately 1.7 to 4.7 times higher risk of a near-miss. Although these data are self-reported, the large sample size and narrow confidence intervals further strengthen our results.

We found no association between OSA severity and near-misses or crashes in women. Although we found that near-misses were related to severity of OSA in Poisson regression analysis for men (Figure 3.2, Table 3.4), this was not significant in multivariable negative binomial analysis (Table 3.7). Crash risk was correlated with AHI but the effect size was small and 'protective' (Table 3.8). This may be due to reporting bias in the very sleepy males or increased awareness of symptoms.
leading to driving avoidance behaviour. Since report bias was low (< 5%), driving avoidance behaviour is the more probable explanation. Our results for AHI are supported by a recent meta-analysis which showed that no definitive conclusion could be drawn regarding a correlation between severity of OSA and crash risk.16

It has long been recognised that there is a poor relationship between the AHI-defined severity of OSA and the degree of daytime sleepiness.19,184,302 It remains unclear why one individual with an AHI of 15 may be sleepy, whereas another may be asymptomatic.303 This poor correlation between AHI and ESS is reflected in our WASHS cohort data (r = 0.18). Since disease severity does not correspond well with symptomatology, it is not surprising that we found an association between risk of near-miss or crash and daytime sleepiness but not the severity of OSA. In our study, we did not find an association between BMI or hypoxaemia and risk of near-miss or crash. Although we found an association between neck circumference and crash risk at the univariable level, after adjusting for other covariates this association did not remain statistically significant.

A marked gender difference in crash risk was found, with women reporting fewer near-misses and crashes than men, particularly for subjects with moderate to severe OSA or an ESS > 10. The preponderance of men with increased near-miss and crash risk in this study is supported by data from the annual report on road crashes in Western Australia, where 75% of fatalities over the past 10 years were male.304 Similar gender differences have been reported in the community-based Wisconsin Sleep Cohort Study.268 It has been postulated that men and women may perceive internal clues to sleepiness differently.268
The association between caffeine use and near-misses was strong (p < 0.001). This provides support for the notion that subjects accurately perceive their increased sleepiness and self-medicate with caffeine in an attempt to remain functional during waking hours. Note that because the question about caffeine use was not asked in relation to driving, it was thus less subject to bias.

**Strengths and Limitations**

The strong relationship between excessive sleepiness and near-misses demonstrated in this study is credible, since it has been shown previously that daytime sleepiness from any cause is associated with increased crash risk. Our finding extends existing knowledge since the most recent systematic review of this topic, which utilised mostly underpowered studies, found no substantial association between ESS scores and crash risk in studies of drivers with OSA. The large number of subjects in our study has provided the power to observe this association following adjustment for confounders. Most studies in this area of research are based on self-reported crashes or near-misses and are thus subject to some bias. The driving questions in this study were asked as part of a general sleep health questionnaire answered at first attendance at the sleep clinic and not in relation to occupation. A high response rate was recorded, but the problem of non-response for near-miss and crash questions remains. Nevertheless, under-reporting of incidents would tend to bias findings towards the null hypothesis and strengthen the positive findings of our study.
A limitation of the data collection method used in our study relates to use of self-reported crash data over the entire driving history for the WASHS cohort, which may have resulted in some recall or report bias. The overall crash rate for patients was compared with police-reported crash rate for the community, and this difference of method may be confounded by measurement bias. Future studies would benefit from the use of objective crash records to improve accuracy. In general, published data supporting increased crashes in OSA are from studies of subjects referred to a sleep clinic. However, Young and colleagues found that their results from a community sample free of clinic bias were comparable in magnitude to the clinic-based studies. Thus impaired drivers with sleep apnoea were not over represented in clinic populations.

The gender differences found in our study may be confounded by driving exposure, which we did not measure. More men than women are occupational drivers and thus at higher risk of crash. Future research should account for this important denominator when considering crash risk in OSA. Gender differences may be attributable to a higher degree of self-awareness of symptomatology in women who self-regulate risk by avoidance of driving.

**Clinical Relevance/Implications of Findings**

These results for our sleep clinic patients can be used to guide clinical management. Our data support the belief that untreated patients with OSA have an increased crash risk but add to this observation that it is those subjects with increased sleepiness regardless of OSA severity who are most at risk. Current Australian Fitness to Drive guidelines have recommended that people should be
advised to avoid or limit driving if they are sleepy, and should not drive if they are at high risk. High-risk individuals include: those with moderate to severe excessive daytime sleepiness (ESS 16-24); those with a history of frequent self-reported sleepiness while driving; and those who have had a crash caused by inattention or sleepiness. These guidelines are particularly relevant for commercial vehicle drivers given that OSA roughly doubles crash risk and close to half of commercial drivers have OSA. Clearly it is important that OSA is diagnosed and the degree of associated sleepiness assessed to expedite treatment and minimise risk. It is known that effective treatment of OSA [e.g., with continuous positive airways pressure] reduces the risk of crash. Recently Sunwoo et al. have shown that a single administration of some objective tests of sleepiness (in particular, the psychomotor vigilance test and the divided attention driving task) can be used routinely in clinical practice to assess sleepiness for diagnostic purposes and for follow-up of outcomes of therapy. This study of commercial drivers provides evidence for a cost-effective routine test that can be applied to occupational drivers to assess risk.

There have been several large studies that have identified similar relationships between sleepiness on the ESS and MVCs in general driving populations. Philip et al. reported that sleepy driving accidents were associated with an odds ratio of 5 where ESS was greater than 15 in a large sample of French drivers. Powell and colleagues conducted a large online survey of drivers in the US and found a significant dose-response relationship between self-report sleepy near-misses and an actual accident. Thus the relationship between
sleepy driving and crash risk is not confined to those with sleep disorders, but may result from volitional sleep restriction.\textsuperscript{308}

It is not yet clear why there is a substantial variation in the degree of sleepiness observed among individuals with equivalent severities of OSA as measured by AHI. Intuitively a patient with severely disordered breathing during sleep with consequent frequent arousals might be expected to be more disabled by sleepiness than one with less sleep disruption. Our findings demonstrate this is not consistently the case and the developing field of research addressing differing neurobehavioral vulnerability to sleep loss may shed light on this subject.\textsuperscript{32} Sleep deprivation studies in normal healthy subjects have shown significant variation between individuals in neurobehavioral deficits with sleep loss, although the effects on each individual are reproducible.\textsuperscript{23} It appears that up to 92\% of the variance in cognitive deficits and subjective sleepiness between individuals may be explained by systematic variability.\textsuperscript{23}

Cluydts \textit{et al.} have suggested that “trait” or person-specific aspects of sleepiness have been largely ignored in sleep wake research.\textsuperscript{309} It appears likely that the sleepiness “trait” for an individual is comprised of several endogenous factors such as their genetic phenotype for sleep propensity (morningness or eveningness), arousal threshold, gender differences in perception, and sensitivity to both stimulant (e.g., caffeine) and sedative (e.g., alcohol) compounds. There will be an interactive effect with environmental or situational factors such as shift work and behaviourally induced insufficient sleep, and consideration must be given to medication use and comorbid medical conditions. Thus the sleepiness phenotype for an individual is likely to be defined by a complex interaction of endogenous and
environmental factors. An individual’s baseline or “trait” level of sleepiness will determine their vulnerability to the additional load of sleep loss imposed by OSA.

In summary, sleep clinic patients with untreated OSA have significantly more crashes than the general population. Our study suggests that sleepiness in untreated OSA is an important contributor to crash risk, particularly in men, underlining the importance of advising such patients, and the community generally, against drowsy driving.

3.7 Appendix

3.7.1 Methods: Multivariable regression analysis

Following is a more detailed summary of the models assessed and risk factors investigated for association with near-misses and crashes. Negative binomial regression was used to assess the impact of a primary risk factor and a number of related factors and potential confounders on the likelihood that subjects would report near-misses or crashes. For all models, there was a significant interaction between the primary risk factor and sex (all p ≤ 0.01); thus the data were stratified by gender.

Four models estimated the association between the primary exposure variables arousal index (ARI) and AHI (both continuous) with near-misses and crashes. These showed no associations with each of two continuous primary exposure variables, with near-misses or crashes at either univariable or multivariable level, and were not further examined. Eight models were fitted to further explore the associations between near-misses and MVCs and the primary
exposure variables of ESS continuous, ESS binary (not sleepy versus sleepy), ESS quartiles and OSA categorical.

A core set of adjustment variables for multivariable regression models (for both near-misses and crashes) were applied: age, NC, time < 90% SaO₂ and alcohol and caffeine consumption. Neck circumference has been shown to have a more significant relationship to severity of OSA than BMI, so NC was the obesity variable used in this analysis. Scrutiny of the data distribution for lowest saturation revealed values lower than 50% arterial oxygen saturation for several subjects. Since these values fall outside the accurate measurement range of the pulse oximeter, these subjects were excluded from analyses. Thereafter the association between lowest saturation and either near-misses or motor vehicle crashes was not significant. On this basis, subsequent analyses were run using the proportion of time spent at an arterial oxygen saturation of less than 90% as the hypoxia variable, and the excluded subjects were returned to the sample. Table 3.9 summarises the exposure and adjustment variables that were significantly associated at the 20% significance level with each of the dependent variables. Note that when testing squared terms, modelling included the linear variable (e.g. \([\text{age} + \text{age}^2]\)) and when testing interactions, linear forms of the variables were included (e.g. \([\text{ESS} + \text{sex} + \text{ESS:sex}]\)).
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<td>6 ESS and Alcohol</td>
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**Note:** The models for near-misses contained different adjustment variables to those for MVCs.

**MVC,** motor vehicle crash; AHI, apnoea hypopnoea index (log transformed); ARI, arousal index (log transformed); ESS, Epworth Sleepiness Scale; ESS severe<sup>b</sup>, binary; NC, neck circumference; OSA, obstructive sleep apnoea presence, defined as AHI>5; SaO<sub>2</sub>, arterial oxygen saturation; #alcohol, standard drinks per week.
4 LITERATURE REVIEW: THE GENETICS OF SLEEP

4.1 FOREWORD

This chapter provides an overview of the literature pertaining to the genetics of sleep. Particular focus is given to the genetic control of sleep-wake regulation and sleep disorders. Rapid progress in the development of genetic analysis techniques has increased our understanding of the genetic molecular mechanism that underpins the circadian rhythm of wake and sleep. Sleep propensity varies in a systematic pattern across 24 hours, but there is substantial variation among individuals in sleep phenotypes such as sleep duration and timing. The substantial body of sleep deprivation research outlined in Chapter 1 highlights the fact that there is significant inter-individual variability in response to sleep loss in terms of performance deficits.\textsuperscript{23} While varied between individuals this response was stable for an individual pointing to trait-like behaviour. Since some people are better able to cope with sleep loss than others, it has been postulated that resilience and vulnerability to sleep loss may well have some genetic determinants. The discovery of genetic variants associated with sleep phase disorders was the precursor to a body of research pursuing genetic variations that may predict individual vulnerability to sleep loss. This has been driven by a growing concern that many people sleep less than 7 hours per night, the minimum sleep duration required by most people to prevent cumulative deterioration in performance on a range of cognitive tasks.\textsuperscript{220,221} For many people this chronic partial sleep loss directly causes significant risks via increased fatigue and sleep propensity, and via cognitive deficits.\textsuperscript{310} This relationship contributes to increased accident risk, and is
a particular issue for people with sleep disorders or shift workers who are further predisposed to sleepiness by their disorder or by work demands. Identifying genetic biomarkers that define individual resilience or vulnerability to sleep loss could help find those most at risk of sleepiness-related accidents and in need of effective countermeasures for sleep loss. For common sleep disorders such as OSA, this will further our capacity to manage people working in safety-critical tasks, especially driving. This literature review has been prepared as a manuscript for publication alongside the genetic analysis presented in Chapter 5.

4.2 INTRODUCTION

Sleep is a complex state serving multiple functions that is characterised by wide variation in timing, duration and structure among normal individuals. Understanding the genetic factors underpinning this variation may lead to clarification of the biological basis and function of sleep and its disorders. Very little is known about the genes that contribute to individual sleep phenotypes, but some of the polymorphic variation in genes that determine different sleep characteristics have been identified over the past 25 years. Like most complex traits or behaviours, sleep is likely to be under the control of multiple genetic and non-genetic (environmental) factors, each of small effect but which combine (and likely interact) to create the phenotype. To identify genes for heritable complex traits requires genetic mapping to correlate genetic variation with phenotype. The past two decades has seen rapid development of genetic analysis techniques to investigate complex traits and disease, as outlined in the following section.
4.2.1 Analytic methods in complex traits

Early human genetic research of rare genetic disorders discovered that some diseases are caused by a single gene mutation (Mendelian disease). These findings helped elucidate the biological pathways and potential medical treatment for monogenic conditions such as cystic fibrosis. Genetic analysis has been very successful in finding the cause of these Mendelian diseases, which follow clear inheritance patterns in families. By contrast, sleep-related traits and disorders are complex phenotypes, that show complex inheritance in the general population. The study of complex traits or diseases involves the study of populations since the phenotypes are highly polygenic, influenced by the environment, and contributed to by genetic variants of individually very low effect. For sleep related phenotypes, the two analytic methods used most often have been candidate gene association and genome-wide association studies (GWAS).

4.2.1.1 Association studies

The goal of association studies is to correlate genetic variation with phenotype in the population, usually represented by the presence or absence of disease or by levels of a disease-related trait. The underlying assumption is that variation that increases the chances of a trait or disease should be enriched in a case sample. The most prevalent of genetic sequence variations are known as single nucleotide polymorphisms (SNPs), with this variation referring to single base differences in sequence between individuals. Early candidate gene studies in sleep compared variants between cases and controls within a single gene of interest with respect to selected SNPs. The other approach to investigation of sleep-related traits (rather than disease) examines mean differences in a
continuous trait conditional on genotype, such as sleep duration. For within case analyses, selected samples with extremes of the distribution can be used to boost power. Candidate gene studies focus on particular genes (or sets of genes) selected on the basis of biological plausibility from prior evidence. Association studies rely on correlation between closely spaced polymorphisms to help narrow chromosomal regions that may harbour mutations. At the population level, this correlation is based upon a phenomenon known as Linkage Disequilibrium (LD). Linkage disequilibrium is influenced by recombination, selection and genetic drift, which must be taken into account during the analysis process. The association of genotype with a disease or trait is a statistical finding that does not necessarily reflect genetic association. Thus far candidate gene studies have met with variable success, the predominant problems being lack of replication and confounding due to population stratification. Therefore, once an association is found, it is important to complete two further steps: replication of the result in other populations, and identification of the genetic variant conferring risk with functional analyses. To support these rigorous requirements, recent advances in interpretation and follow-up techniques have validated ongoing use of the candidate gene association approach. Where there are plausible genes (loci) identified that may associate with a well-established physiological basis (outcome), candidate gene association studies have a role to play. However, lack of replication of past association studies has led researchers to seek a more unbiased survey of the entire genome, and thus the GWAS has evolved to identify genetic associations for complex traits.
4.2.1.2 Genome-wide association study

Advances in quantitative genetics have enabled the study of common genetic variation in conjunction with disease status or an observable trait, without having a priori knowledge of functionality of variants or their position in the genome. Genome-wide association studies (GWAS) are hypothesis free and do not require a plausible link between the loci and the outcome. A GWAS is the gold standard method for testing the magnitude of any correlation between common SNPs and a complex phenotype (e.g. sleepiness or diabetes). These studies have been revolutionary in that they allow the comparison of large numbers (typically 1,000 or more unrelated individuals) of cases with the complex phenotype and an equivalent number of suitable controls (e.g. population based controls). The whole genome is ‘screened’ using densely genotyped SNPs (> 100,000 to 5 million markers) to look for associations between common SNPs and the outcome measure. Many statistical tests are conducted with this method (at least one per SNP) so a more stringent p-value must be applied to represent significance, usually in the order of $p < 5 \times 10^{-8}$. This multiple testing problem necessitates working with very large datasets. Once SNP associations are found, the challenge remains to determine the impact of SNP variants on the trait or disease. SNP variation at a site does not imply a functional role, and SNPs can be located within or outside the coding and regulatory sequences of the gene. A SNP may be a marker of a functionally relevant variant rather than a causal site. In order to clarify the role of an associated SNP identified by GWAS, the findings must be replicated in multiple populations (ideally of different ethnicity), and functional studies conducted to assess the impact of the variant on a disease or trait. The best evidence of causality would be to show that manipulating the gene affects
phenotype *in vivo* in a model organism such as the mouse. To summarise, a simplified approach to GWAS involves seven steps: sample collection, genotyping, quality control, association testing, population stratification assessment, replication and functional studies.

To date over 400 GWAS studies of complex traits and diseases have been published, the results of which have led to the development of clear guidelines for the correct methodology and interpretation of these data. By contrast, there have been relatively few GWAS studies of sleep disorders and sleep-related phenotypes (< 20), so relatively few sleep genes have yet been defined. The following sections provide an overview of the current state of knowledge of the genetics of sleep pertinent to this thesis.

### 4.3 Genetics of Sleep

Evidence is accumulating that many aspects of normal sleep-wake regulation are in part genetically controlled, but it is unsurprising to find that there is no single sleep gene. Sleep is a complex state defined by a recurrent behavioural state timed across a 24-hour clock, with characteristic EEG changes and responses to deprivation. Thus sleep behaviour will be controlled and influenced by many genes and environmental factors interacting to create phenotypes. Research into the genetics of sleep has predominantly focussed on two areas: the genetics of sleep disorders and diseases that result in sleep disturbance (categorical phenotypes), and the genetics of sleep-wake regulation (continuous phenotypes).
4.3.1 Genetics of sleep disorders

The official catalogue of sleep disorders covers a spectrum of conditions both rare and common, in addition to disorders with a more environmental aetiology such as jet lag, shift work and inadequate sleep hygiene. An example of a sleep disorder with simple genetic inheritance is the rare Mendelian condition of Fatal Familial Insomnia. This condition is genetically determined by a mutation that alters a prion protein and results in disease with little environmental influence. By contrast, most sleep disorders are categorised as complex genetic diseases. Much progress has been made in the understanding of narcolepsy (less rare with ~1/2000 cases) via studies of families and twins. Twin studies provide a tool to assess the relative contribution of genetics and environment in sleep disorders. In narcolepsy, the twin concordance rate is ~ 30%, suggesting that both genetic and environmental contributions influence the expression of the disorder.

Many early associations for sleep disorders were found through the candidate gene approach where disorders had known physiological bases. Early work in Drosophila and mice identified gene mutations that correlate with changes in circadian sleep-wake cycle length, and a model of its molecular mechanism followed (Section 4.2.1). Many candidate gene studies have targeted circadian genes, based on the detailed understanding of the molecular underpinnings of the circadian clock and the connection with sleep regulation in humans and model organisms (Section 2.3.2). With respect to humans, circadian genes have been targeted due to the influence of the circadian clock on sleep behaviours. Thirty years after the pioneering work on Period (PER) gene mutants in Drosophila, it was discovered that familial advanced sleep phase syndrome is
caused by mutations in human clock-related genes. A mutation in \textit{PER2} results in individuals having a 4-6 hour phase advance in sleep and wake times, on a background of normal sleep architecture.\textsuperscript{329} Subsequently, several associations between clock genes and circadian rhythm disorders have been found (Table 4.2: Period genes).\textsuperscript{330} The success of this approach is due to the clear understanding of the molecular pathways underlying circadian rhythm and explicit definition of phenotypes.\textsuperscript{331}

Most recently GWAS have shed light on the genetics of common complex traits such as restless legs syndrome (5-10% population).\textsuperscript{27} It was known that restless legs syndrome had a strong genetic component by high concordance in monozygotic twins (83%), and up to 60% of cases reporting affected family members.\textsuperscript{332} However attempts to identify specific genes by linkage analysis in families failed to find either mutations or genes. GWAS were successful in reporting four loci associated with restless legs syndrome.\textsuperscript{333} Each at-risk allele of the gene \textit{BTBD9} is associated with a 13% decrease in ferritin. There is a well-described association between RLS and lower iron body stores, so these results have led to the development of a hypothesis around the underlying regulation and sensory perception for this disorder.\textsuperscript{332} This knowledge can then be translated to inform study design for future GWAS and to develop treatments for the condition.

The cases in this thesis have OSA, another complex common disorder. To date there has been limited success defining the genetics of OSA, although family studies have shown that inherited factors account for about 40% of the risk of OSA.\textsuperscript{334} Familial aggregation of cases of OSA has been reported in several studies,
with a major difference in heritability between blacks and whites found in the Cleveland Family Study. Genetic and physiological data to date suggest that traits most likely to affect predisposition to OSA are related to the anatomy of the oropharynx, ventilatory control and obesity. Conceptually, OSA can be thought of as a complex disease expressed once a certain level of susceptibility is exceeded. Susceptibility relates to the predisposition for repeated upper airway collapse and individual study of intermediate phenotypes, such as craniofacial morphology, ventilatory control and sleepiness may yield insights. Further clarity on the genetic epidemiology of OSA will be gained by GWAS of large well-phenotyped cohorts facilitated by international collaborations. The primary symptom associated with OSA and focus of this thesis is excessive sleepiness, a continuous trait. The genetic epidemiology of excessive sleepiness in OSA has been the subject of limited research to date, although it is increasingly considered an important health problem contributing to increased risk of accidents, psychosocial morbidity and poor quality of life. Excessive sleepiness is heritable with estimates from recent twin studies in the range of 38 to 48%. Although excessive sleepiness is recognized as a heritable trait, the genetic basis is largely unknown. Elucidation of the genetic basis will benefit from clear definition of intermediate phenotypes, and for individuals with OSA in particular, attention to sleep phenotyping will be crucial. The multiple genetic, environmental and physiological factors that contribute to an individual sleep phenotype are considered in the following section.
4.3.2 Genetics of sleep-wake regulation

In humans several features of normal sleep are known to be heritable. Evidence of a genetic component in the regulation of human sleep is borne out by heritability of sleep traits, identification of specific genetic polymorphisms that affect these traits and the existence of familial sleep disorders. Traditionally twin studies have been used to estimate heritability of a trait by comparing monozygotic (MZ) pairs (identical genetically) with dizygotic (DZ) twins (who share 50% of genes). Heritability estimates of sleep phenotypes vary between approximately 20-40% for habitual sleep duration, to over 90% for the spectral characteristics of the EEG in non-REM sleep. Sleep timing is an integral component of the sleep-wake cycle (Section 2.3.2). Twin studies suggest a genetic component in diurnal types (chronotype), with preference for timing to bed and waking estimated to be about 50% heritable. Chronotype can be measured using a simple questionnaire and has a genetic basis but is also influenced by light exposure, age and gender.

Although a proportion of several sleep traits is heritable, there is wide individual variation in duration, timing and structure. Extremes of some phenotypes can extend into the realm of sleep disorders, examples being advanced and delayed sleep phase disorders which represent the boundaries of variation in sleep timing and diurnal preference. Sleep genetic studies have used the candidate gene approach to explore associations between clock genes and sleep phase disorders in cases versus controls. An identified genetic determinant of abnormal sleep timing is a mutation of the key clock gene Period2 (PER2) which results in family members suffering advanced sleep phase syndrome. Very early
sleep timing (4-6 hours advance) alters the timing of the sleep period, but sleep structure is normal. By contrast, there are polymorphisms in both a Clock gene\textsuperscript{347} and a \textit{PER3} gene\textsuperscript{348} that are associated with delayed sleep phase syndrome. These early studies focussed on circadian genes due to the well-known connections between circadian control and sleep regulation in animal models and humans.\textsuperscript{349} The core set of genes (clock genes) involved in the generation of circadian rhythmicity are known.\textsuperscript{331} In the following section the molecular mechanism of clock genes is described to provide the framework for the rationale of the selection of these genes for analysis in this project.

4.3.3 \textbf{Clock genes}

A clear, genetic, molecular mechanism regulates circadian rhythm over a 24 hour cycle.\textsuperscript{331} The circadian sleep-wake cycle is based on a complex feedback loop of genetic transcription first elucidated in \textit{Drosophila},\textsuperscript{327} but since shown to be similar in mice and humans (Table 4.1).\textsuperscript{349} Due to the high degree of conservation across model systems, the molecular basis for circadian rhythms has been well characterised.\textsuperscript{27}

\textbf{Table 4.1.} Mammalian genes that influence circadian rhythm and their close counterparts in the \textit{Drosophila melanogaster} fruit fly\textsuperscript{349}

<table>
<thead>
<tr>
<th>Mammalian gene</th>
<th>\textit{Drosophila} gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Clock} (\textit{CLOCK}\textsuperscript{a})</td>
<td>\textit{Clock}</td>
</tr>
<tr>
<td>\textit{Period} 1,2,3 (\textit{PER1, PER2, PER3}\textsuperscript{a})</td>
<td>\textit{Period}</td>
</tr>
<tr>
<td>\textit{Cryptochrome} 1 and 2 (\textit{CRY1, CRY2}\textsuperscript{a})</td>
<td>\textit{Cryptochrome}</td>
</tr>
<tr>
<td>\textit{BMAL1} (\textit{ARNTL1}\textsuperscript{a})</td>
<td>\textit{Cycle}</td>
</tr>
<tr>
<td>\textit{Timeless} (\textit{TIMELESS}\textsuperscript{a})</td>
<td>\textit{Timeless}</td>
</tr>
<tr>
<td>\textit{CK1δ and CK1δ} (\textit{CSNK1E}\textsuperscript{a})</td>
<td>\textit{Doubletime}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Currently approved gene symbol\textsuperscript{350}
Circadian rhythms are regulated by a core set of clock genes that modulate molecular feedback loops, in which proteins regulate their own expression at the cellular level (Table 4.1). The various elements that contribute to sleep-wake regulation, including the role of the clock genes are illustrated in Figure 4.1. The circadian pacemaker and sleep homeostat interact to regulate the sleep-wake cycle, which is also affected by social factors such as work schedule (Section 2.3.2). The sleep-wake cycle drives the sleep homeostat and feeds back onto the circadian pacemaker, which is synchronised by the light-dark cycle. The clock genes generate circadian rhythms, some of which also affect sleep homeostasis (Section 4.3.4).

Figure 4.1. Elements of sleep-wake regulation. Modified from Dijk et al.

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Much more is known about the genetic bases of circadian rhythm than the sleep homeostat, due to the extensive work conducted in mice and Drosophila. In humans, the candidate gene approach has found associations between clock genes and sleep phase disorders. Over the past ten years a large body of work has explored the association between period genes (one group of the clock genes), normal sleep and sleep disorders.

4.3.4 Period genes

The Period gene family (PER1, PER2 and PER3) is central to the control mechanism that generates circadian rhythm. This role has been demonstrated in mice, and recent research has suggested a sleep homeostat involvement of period genes in sleep-wake regulation. In humans, the role of period genes has been assessed by analysing the consequences of natural variation in polymorphisms and mutations of these genes (Table 4.2). Polymorphisms in human PER2 and PER3 genes associate with clear phenotypic differences, and studies have explored associations for clearly defined phenotypes such as morning and evening types with sleep phase disorders. Toh et al. linked a mutation in PER2 with the circadian sleep disorder known as familial advanced sleep phase syndrome. In the same year (2001), Ebisawa et al. linked a missense polymorphism (PER3) to delayed sleep phase syndrome. Soon after, Archer et al. reported a variation in the coding region of the PER3 gene was linked to delayed sleep phase syndrome and extreme diurnal preference. This group was the first to apply a prospective study design focussing specifically on a length-polymorphic repeat region composed of either 4 or 5 units in the coding region of PER3.
In a pivotal prospective study in 2007, Viola et al. explored the contribution of this repeat polymorphism by selecting healthy participants only on the basis of their *PER3* genotype. This primate-specific variable-number tandem-repeat (VNTR) polymorphism in *PER3* contains a 54-nucleotide unit, which is repeated four or five times. Individuals were genotyped in order to select matched pairs who were homozygous for the short allele (*PER3*<sup>4/4</sup>) and long allele (*PER3*<sup>5/5</sup>).

The study investigated the functional consequences of this polymorphism for sleep and circadian physiology plus waking performance. The results showed that *PER3*<sup>5/5</sup> homozygotes had a shorter sleep latency, with profound differences in EEG markers of sleep homeostasis after sleep deprivation, spending 50% more of their time in deep sleep. The behavioural consequences of this deprivation were much greater deficits in cognitive function in the *PER3*<sup>5/5</sup> homozygotes. This was the first of a series of studies to provide evidence for the hypothesis that there is at least some cross-talk between the circadian process and the sleep homeostat.

The genotype-dependent differences in response to sleep deprivation were of particular interest, and initiated intensive investigation of a proposed resilience or vulnerability to sleep loss mediated by the *PER3* VNTR polymorphism (Table 4.2). Resilience or vulnerability to sleep loss has important implications for fatigue risk management in the workplace, and for the symptomatology of sleep disorders, prompting research efforts to identify reliable predictors.
<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Reference</th>
<th>Nearest Gene</th>
<th>SNP</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katzenberg 1998</td>
<td>347</td>
<td>Clock</td>
<td>rs1801260</td>
<td>A Clock polymorphism associated with human diurnal preference</td>
</tr>
<tr>
<td>Toh 2001</td>
<td>329</td>
<td>Period2</td>
<td></td>
<td>PER2 gene associated with familial Advanced Sleep Phase Syndrome</td>
</tr>
<tr>
<td>Ebisawa 2001</td>
<td>355</td>
<td>Period3</td>
<td></td>
<td>A missense polymorphism linked to delayed sleep phase syndrome</td>
</tr>
<tr>
<td>Archer 2003</td>
<td>348</td>
<td>Period3</td>
<td>rs1801260</td>
<td>VNR polymorphism of PER3 linked to extreme diurnal preference</td>
</tr>
<tr>
<td>Mishima 2005</td>
<td>359</td>
<td>Clock</td>
<td>rs1801260</td>
<td>The 3111C allele of the Clock gene associated with eveningness and delayed sleep timing</td>
</tr>
<tr>
<td>Carpen 2005</td>
<td>360</td>
<td>Period2</td>
<td></td>
<td>PER2 gene associated with morning preference</td>
</tr>
<tr>
<td>Viola 2007</td>
<td>356</td>
<td>Period3</td>
<td>rs57875989</td>
<td>PER3 polymorphism predicts sleep structure and waking performance</td>
</tr>
<tr>
<td>Goel 2009</td>
<td>361</td>
<td>Period3</td>
<td>rs57875989</td>
<td>PER3 VNTR polymorphism predicts cumulative sleep homeostatic but not neurobehavioural vulnerability to partial sleep deprivation</td>
</tr>
<tr>
<td>Archer 2010</td>
<td>362</td>
<td>PER3 VNTR</td>
<td>rs279687</td>
<td>SNPs in the PER3 promoter associate with diurnal preference and delayed sleep phase syndrome</td>
</tr>
<tr>
<td>Lo 2012</td>
<td>363</td>
<td>PER3 VNTR</td>
<td>rs57875989</td>
<td>Greater impairment in PER35/5 carriers during early morning hours under sleep deprivation in several cognitive tests</td>
</tr>
<tr>
<td>Ojeda 2013</td>
<td>364</td>
<td>Period2</td>
<td>rs934945</td>
<td>PER2 and PER3 SNPs investigated for association with diurnal preference</td>
</tr>
<tr>
<td>An 2014</td>
<td>365</td>
<td>Period3</td>
<td>rs2640909</td>
<td>Diurnal preference not associated with the PER3 VNTR polymorphism in Han Chinese pilots</td>
</tr>
<tr>
<td>Maire 2014</td>
<td>366</td>
<td>Period3</td>
<td>rs57875989</td>
<td>Physiological markers of sleepiness are more pronounced in PER35/5 carriers</td>
</tr>
<tr>
<td>Drake 2015</td>
<td>367</td>
<td>Period3</td>
<td>rs57875989</td>
<td>PER3 VNTR polymorphism is associated with sleepiness and maladaptive circadian phase in night-shift workers</td>
</tr>
</tbody>
</table>

PER2, Period2 gene; PER3, Period3 gene; VNTR, variable-number tandem-repeat; SNPs, single nucleotide polymorphisms; PER35/5, homozygote of the 5 repeat Per3 VNTR polymorphism
4.3.5 Period genes: Vulnerability to sleep loss

In 2004 Van Dongen et al. reported that there are considerable, stable individual differences in performance impairment due to sleep deprivation (Section 2.7.5). These differences were postulated to constitute a trait since the response to sleep loss was stable within an individual. Subsequent to this finding there has been a concerted effort to find candidate predictors of individual resistance or vulnerability to cognitive impairment due to sleep loss. A few genetic polymorphisms have been found to modulate individual vulnerability to sleep deprivation. Most research has centred on genes related to the regulation of sleep, sleep homeostasis and clock genes involved in circadian rhythmicity, in particular the clock gene PER3. A twin study conducted by Kuna et al. found the estimated heritability of vulnerability to sleep loss to be 83.4% providing support for a strong genetic basis. Certain genes have been implicated to explain the large individual differences in vulnerability to sleep loss, but the effect sizes are likely to be small. Thus for any given cognitive outcome, differential vulnerability to sleep loss is likely to be a polygenic trait, with complex interactions between the various neurobehavioural domains.

It has been advocated that accounting for individual differences in traits that control sleep-wake regulation and vulnerability to sleep loss would enhance our understanding of the diagnosis and treatment of sleep disorders such as OSA. Obstructive sleep apnoea is a sleep disorder that disrupts the sleep-wake balance resulting in neurocognitive deficits due to repetitive hypoxia and sleep fragmentation (Section 2.6.4). Subjects with OSA display very similar cognitive
deficits to those of sleep-deprived healthy individuals. It may be that findings from sleep deprivation research can be applied to postulate upon the source of variable symptomatology and neurobehavioural deficits in OSA. Specifically, differential vulnerability to sleep loss mediated by genetic variation may play a role in OSA symptomatology (Section 2.6.2). Reduced costs and enhanced methodologies have led to further attempts to characterise the genetic profile of sleep-related traits, and a summary of GWAS conducted to date follows.

4.3.6 Sleep phenotypes: Evidence from genome wide association studies

The first GWAS of sleep related phenotypes was performed by collecting questionnaire data on sleep habits and sleepiness within an existing cohort (Framingham Heart Study offspring cohort). The study design allowed linkage analyses (family-based) for heritability in addition to association analyses by GWAS (749 individuals). The aims of the study were two-fold; to replicate previous findings of heritability for sleepiness, diurnal preference and sleep duration, and to conduct genome-wide linkage and association studies of these traits. Consistent with past studies, significant heritability (proportion attributable to genotype) of sleepiness (0.29, p < 0.001), usual bedtime (0.22, p < 0.01) and sleep duration (0.17, p = 0.02) was confirmed, although the estimates were lower than found in twin studies. Several genetic loci with suggestive linkage to these traits were identified, including linkage peaks containing circadian clock-related genes. In addition, population-based association testing identified eight SNPs associated with the sleep phenotypes with p < 10^-5. The lowest p-value reported (p = 2.5*10^-8) was for an association between sleepiness and a SNP on chromosome 5. This SNP is located in the non-coding region of the (PDE4D) gene, so no direct
functional role is indicated. However, since some of the function and expression patterns of \textit{PDE4D} in the brain are known, the investigators proposed that its effect on intracellular levels of cyclic adenosine monophosphate (cAMP) might mediate sleepiness. The primary limitations of this study were lack of power and definition of the sleep phenotype by questionnaire rather than objective means, e.g. sleep study. Therefore, the results require replication. Nevertheless, the approach adopted by Gottlieb \textit{et al.} has provided significant cost savings by analysing sleep phenotypes in an existing cohort. The study has applied current genetic epidemiology methodologies to the study of common sleep and circadian phenotypes and identified genes for further study not previously implicated as having a role in human sleep.

Since 2007 there have been four further GWAS conducted to further understanding of sleep and circadian phenotypes. In 2013 Byrne \textit{et al.} conducted a GWAS in > 2000 Australian twins to identify common variants that influence sleep traits, and to replicate the findings of Gottlieb \textit{et al.} No SNPs reached the GWAS threshold for significance, however some associations were identified in plausible candidate genes. The strongest association found ($p = 1.3\times10^{-6}$) was for a gene previously shown to have an association with bipolar disorder. The \textit{CACNA1C} (calcium channel, voltage-dependent, L type, alpha 1C subunit) gene showed evidence for association with sleep latency and quality. The association did not replicate in an independent sample, nor were the findings of Gottlieb \textit{et al.} replicated except for one of 34 associations. Nevertheless past work has suggested a link between sleep disturbance and mood disorders, with some associations between circadian genes and mood disorders reported. Thus \textit{CACNA1C}
represents a biologically plausible candidate gene for sleep phenotypes and warrants replication efforts.

A comprehensive evaluation of genetic variants associated with sleep duration was reported by Allebrandt et al. in 2013.\textsuperscript{375} Their study explored the hypothesis that too little and too much sleep is associated with several health deficits and general mortality.\textsuperscript{376} This strong study was well-powered (n = 4,251, European consortium) utilising both a discovery cohort and replication sample, followed by a functional study in \textit{Drosophila}. The group identified one SNP in the \textit{ABCC9} gene, an adenosine triphosphate (ATP)-sensitive potassium channel that associated with usual sleep duration and explained about 5\% of the variance for this phenotype. This result was confirmed in the replication sample when adjustment for season and chronotype was made. A functional analysis in \textit{Drosophila} found knockdown of \textit{ABCC9} results in shortened sleep duration, thus corroborating the notion that this locus is relevant for the modulation of sleep across species.\textsuperscript{375} The findings of this study have implications for the regulation of sleep duration, with the underlying mechanisms yet to be elucidated.

Later in 2013 Parsons et al. attempted to replicate the most significant findings from the two larger GWAS\textsuperscript{372,375} using a large British twin and sibling sample (n = 952).\textsuperscript{377} The association of a genetic variant in the \textit{CACNA1C} gene with sleep quality was replicated,\textsuperscript{377} thus adding support for the involvement of calcium channels in the mechanism regulating sleep function. The association between \textit{ABCC9} and sleep duration\textsuperscript{375} was not replicated but a significant association between this variant and depressive symptoms was reported.\textsuperscript{377} This evidence
warrants further exploration with functional studies to pursue the relationship of these two genes with sleep and depression.

Most recently, Gottlieb et al. (the CHARGE Consortium) have conducted the largest GWAS for usual sleep duration to date, with 18 population-based cohorts (n = 47,180) of European ancestry.\textsuperscript{378} Two novel loci with genome-wide significant (p < 10\textsuperscript{-8}) association to self-reported usual sleep duration were identified in these discovery cohorts. One of the loci was replicated in a sample of African Americans, suggesting a true effect supported by cross-racial replication. This GWAS included multiple SNPs previously associated with sleep duration or chronotype, and other core mammalian clock genes, such as \textit{ABCC9}, \textit{PER2}, \textit{PER3} and \textit{CLOCK}. However, there was no evidence of association of any of these SNPs with usual sleep duration. Thus past findings of association for \textit{ABCC9} and \textit{CLOCK} by Gottlieb\textsuperscript{25} and Allebrandt\textsuperscript{379} have not been replicated despite this study being greater than ten-fold larger than the two previously published GWAS of sleep duration.

To summarise, some progress has been made with GWAS analyses of sleep-related phenotypes and traits. However limited success reinforces that complex traits are contributed to by multiple genetic factors of individually low effect, probably interacting substantially with environmental factors.\textsuperscript{314} For sleep traits, some environmental factors likely to be influential are social and variable (such as shift work, jet lag and late nights) while others (such as sleep disruption due to a sleep disorder) are more fixed and vary from one person to another.\textsuperscript{378} This variability across sleep traits highlights the challenge of sleep phenotyping and careful variable selection for future genetic analyses.
4.3.7 The sleepiness phenotype in OSA

Across 24 hours, the level of sleepiness in individuals is modulated by a combination of circadian (time of day) and homeostatic (duration of time awake) factors (Section 2.3.2). The overall rise and fall morphology of sleep homeostasis is relatively stable across humans. However, sleep deprivation studies have shown that there is large variability between individuals in initial sleep pressure and in sensitivity and responsiveness of the sleep homeostat. This sleep pressure variation has been demonstrated in extended wakefulness studies. Those who cope best are evening types and short sleepers (those who habitually sleep < 6 hours), with the differences reflected in the steepness and the timing of subjective sleepiness. Similar variability is seen in the rhythmic expression of sleepiness with respect to the period, phase and amplitude of the circadian oscillator. Forced desynchrony research protocols have shown that both homeostatic and circadian processes influence sleepiness and performance. Long sleepers have a longer biological night as represented by longer nocturnal intervals of plasma melatonin and cortisol, core body temperature and subjective arousal. There is a two hour delay in the phase of the circadian rhythm sleepiness between morning and evening types, as measured by core body temperature nadir. Thus the two process model of sleepiness predicts important fluctuations in the ‘sleepiness’ state across the 24 hour period, but does not take into account an individual’s stable characteristic level of sleepiness or ‘trait’ level.
Recent work has shown that individual differences in sleep traits are also related to genetic factors. To describe individual differences in aspects of sleep, it is necessary to consider both genotypic and phenotypic features of sleep. A pivotal study examining performance impairment consequent to sleep loss concluded that systematic inter-individual differences in neurobehavioural deficits from sleep loss constituted a differential vulnerability trait (Section 2.7.5: Individual variability in human sleep). There has been a surge of research activity directed toward the identification of determinants of this vulnerability and resistance to sleep loss. This has been facilitated by a large body of work demonstrating that the master clock in the suprachiasmatic nucleus is activated by the clock and period genes that mediate circadian periodicity (Section 4.2.3 Clock genes). Polymorphisms in clock genes affect diurnal preference, sleep timing and sleep structure (Section 4.2.4). Viola et al. were the first group to show that PER3 genotype-dependent differences exist for individuals with respect to sleep and circadian physiology, and waking performance. This evidence of a genetic determinant of a differential response to sleep loss has implications for fatigue risk management in healthy individuals, and for the symptomatology and treatment of sleep disorders.

While excessive daytime sleepiness is the most common symptom associated with OSA, complaints of sleepiness are absent in many individuals. There is substantial variation in sleepiness symptomatology reported by patients relative to the severity of OSA. The source of this variation is unclear, but it may be that “trait” or person-specific aspects of sleepiness proposed by Cluydts et al. in 2002 play a role. Daytime sleepiness (as measured by subjective scores such as the
ESS) is a continuous trait with normal distribution in the general population. As such, one would expect that the sleepiness trait is dependent on minor but common polymorphisms in multiple genes. Genetic association studies of sleep phenotypes to date have reported some associations but few have been replicated (Table 4.2). Most recently Kripke et al. took the approach first utilised by Gottlieb et al. in 2007 and prospectively recruited sleep clinic patients for genetic association analyses of sleep and sleep disorder phenotypes. The sleepiness variant reported by Gottlieb et al. was not replicated, but novel associations of polymorphisms with several sleep phenotypes were reported. Although the sample size was modest (n ~ 700 patients), a strength of this study was the accurate sleep phenotyping of cases with polysomnography. Thus the single reported genetic association for daytime sleepiness is yet to be replicated. As shown for other complex traits, it is likely that the sleepiness phenotype is comprised of multiple genetic factors of individually low effect, most of which are yet to be elucidated. To gain a complete understanding of the mechanisms underlying sleepiness in healthy individuals and those with OSA, consideration must be given to the genetics underpinning both sleep homeostasis and circadian rhythm as state systems. In addition the individual components of an individual sleepiness trait will include factors such as usual sleep duration and sleep timing (morning and evening preference), and substantial environmental influences. Identification of genetic biomarkers of differential vulnerability to sleep loss will facilitate management of excessive sleepiness due to common disorders such as OSA.
4.3.8 Summary

Expanded understanding of sleep has shown that it plays a central role in health and disease, and as such, further knowledge about the genetic regulation of normal sleep and sleep disorders would benefit public health. Sleep is a complex process, and it is clear that genetic factors underlie both normal sleep and sleep disorders. Rapid advances in genetic analysis techniques have uncovered some of the genetic bases of sleep processes, including homeostatic response, circadian behavioural variation and numerous sleep disorders. Many association studies have been published (candidate gene and GWAS), but relatively few associations have been replicated. One review reported that of 166 putative associations studied three or four times, only six were consistently replicated. Why have the findings of well-designed association studies and GWAS not been replicated? Complex traits are defined by multiple genes of very low individual effect and interactions with the environment, so most studies have been underpowered. Results of a recent large GWAS (GIANT consortium; 250,000 individuals) found of 32 loci showing genome-wide association with body mass index, only four variants explained > 0.07% of the variance in body mass index, and the remaining 30 variants explained 0.03% variance each. Another reason for failure to replicate may relate to imprecise phenotype of the correlate under investigation. Studies of sleep phenotypes often use self-report data collected by questionnaire, (e.g. sleep duration) which may not have the precision required for genetic association studies. Sleep behaviours are substantially influenced by environmental factors, thus rigorous phenotyping of sleep variables is crucial. Due to the acknowledged limitations of past GWAS analyses, candidate gene association studies have come to the fore to study genetically complex traits, and
may be suitable for sleep-related phenotypes.\textsuperscript{312} In the field of clinical sleep medicine and research, many thousands of polysomnography studies are conducted providing the opportunity to collate well-phenotyped cohorts of individuals to investigate normal genetic variation in conjunction with disease status and symptomatology. In parallel with the collaboration of consortia, new sequencing technologies are under development for both whole-genome sequencing and exomic sequencing.\textsuperscript{321} In the future the combination of large well-phenotyped cohorts and new sequencing technologies has the promise to unlock more of the genetic bases of sleep traits and disease. Developing an understanding of the genetic factors that underlie a phenotype or disorder can contribute to knowledge of the biological basis and expression of the condition.\textsuperscript{312} The study reported in Chapter 5 has analysed the association of known sleep-related variants (\textit{PER2} and \textit{PER3}) with sleepiness in a well-phenotyped cohort of individuals with OSA.
5 A NOVEL ASSOCIATION OF POLYMORPHISMS IN THE CIRCADIAN CLOCK PERIOD3 GENE WITH SLEEPINESS IN OBSTRUCTIVE SLEEP APNOEA

5.1 Foreword

The study described in Chapter 3 demonstrates: (i) that untreated OSA is associated with an increased risk of self-reported near-misses in men and women, and an increased risk of MVCs in very sleepy men, and (ii) that there is a strong association between excessive daytime sleepiness and increased report of near-misses. Thus, our data support the observation that it is those patients with increased sleepiness regardless of OSA severity that are most at risk. This chapter utilises a within-cohort analysis of well-phenotyped OSA patients (sampled from WASHS) to investigate the relationship between variation in Period (PER) genes and sleepiness in OSA patients. The literature review of the genetics of sleep (Chapter 4) illustrates that there have been few studies of sleep phenotypes to date, and none that have focussed specifically on sleepiness in untreated OSA patients. Some genes associated with sleep phenotypes have been identified by GWAS, but few have been replicated. Previous research in healthy subjects has suggested a role for PER3 variants in determining sleep/wake regulation, sleep structure and differential vulnerability to sleep loss.\textsuperscript{356} Genotype-dependent differences in sleepiness and vigilance in response to sleep loss were found. There are parallels between the cognitive deficits seen in response to sleep loss in healthy subjects and those of OSA cases whose sleep is disrupted. In addition, there is wide inter-individual variability that is trait-like, and postulated to be
determined in part by genetic factors. In OSA, patient sleepiness may be highly variable despite comparable OSA severity, and the basis of this variation is not well understood. This chapter builds on existing sleep-wake genetic research by examining the association of PER2 and PER3 variants with excessive sleepiness in an OSA cohort. The content of this chapter has been prepared as a manuscript for publication.

5.2 ABSTRACT

**Background:** Excessive sleepiness and cognitive impairments are common debilitating symptoms in OSA patients. Sleepiness can be influenced by many factors, including age, sex, shift work, medications, and lifestyle factors; however the role of genetic variation has been little investigated to date.

**Study objectives:** To investigate the association of known sleep-wake regulation candidate genes (Period 2 and Period 3) with “sleepy” phenotypes in subjects with OSA from the Western Australian Sleep Health Study (WASHS).

**Design:** Within-case analysis

**Methods:** Participants were 1,301 OSA cases from the WASHS who completed a questionnaire, provided a DNA sample and had overnight polysomnography. Genetic association analyses between fifty single-nucleotide polymorphisms (SNPs) in two sleep-wake regulation Period genes (PER2 and PER3) and sleepiness were performed, controlling for relevant covariates. All linear and binary logistic regression models were adjusted for age, sex, obesity and caffeine and alcohol use. Apnoea-hypopnoea index (AHI) and hypoxia indices
(lowest arterial oxygen saturation \([\text{SaO}_2]\) and time spent at less than 90\% \text{SaO}_2), but not sleep duration were associated with Epworth sleepiness score (ESS) or dozing and were therefore tested in all models.

**Results:** Increasing age, obesity and caffeine use were associated with an increased risk of an elevated ESS. Three \textit{PER3} SNPs were associated with low somnificity (risk of dozing in an alerting situation, all \(p \leq 0.001\)), after adjusting for AHI and a SNP*AHI interaction. Genotype-dependent differences related to severity of OSA were apparent in dozers versus non-dozers. Cases who were homozygous for the minor allele (\(\text{AA}\)) of rs697693 are at risk of dozing for less severe OSA (a lower AHI of 24) than either major homozygotes (\(\text{GG}\)) or heterozygotes (\(\text{AG}\)).

**Conclusion:** This study found novel associations between \textit{PER3} variants and risk of dozing in OSA cases, mediated by an interaction with severity of OSA. Genotype-dependent differences in response to sleep disruption are suggestive of differential vulnerability to sleep loss mediated by genetic determinants. Further studies are required to confirm this finding.
5.3 INTRODUCTION

Excessive sleepiness is the most common daytime symptom associated with the highly prevalent respiratory sleep disorder obstructive sleep apnoea (OSA). However sleepiness is absent in many patients, and the relationship between OSA severity and sleepiness is weak. Disease severity is generally classified by the metric of apnoea hypopnoea index (AHI), the number of breathing pauses per hour of sleep. Some large studies have shown a statistically positive relationship between AHI and sleepiness, but the correlation rarely exceeds a value of 0.4. Thus disease severity does not correspond well with symptomatology, and it remains unclear why one individual with moderate OSA may be excessively sleepy while another is asymptomatic. In addition to sleepiness, OSA sufferers experience impaired cognitive function and are at increased risk of motor vehicle or occupational accidents. Thus sleep physicians are faced with a management dilemma when diagnosing and treating people employed in safety-critical industries. To manage the risk of individuals performing safety-critical tasks such as driving, better tools are needed to measure OSA-related cognitive impairment, especially daytime sleepiness.

There is growing concern about the prevalence and consequences of sleep loss in the community since 20% of adults report poor or inadequate sleep, about half of which is attributable to common sleep disorders like OSA and insomnia. In addition to common sleep pathologies, the proportion of people curtailing their sleep due to lifestyle and shift work is increasing. Surveys show that a large proportion of people fail to achieve the minimum sleep duration of seven hours
per night required to prevent cumulative deterioration in performance on a range of cognitive tasks. For the majority of people, sleep loss directly causes significant disability and risk because of increased fatigue, sleep propensity, and deficits in cognitive function. These deficits represent a significant public health concern, and thus sleep loss outcomes have been widely researched with sleep deprivation studies. These studies have shown that sleep loss in humans results in a range of neurocognitive deficits, such as declines in vigilance, working memory, and executive function.

The cognitive decline associated with sleep loss has been demonstrated in carefully controlled studies of both total and partial sleep deprivation. Although both forms of sleep deprivation result in cognitive deficits, there is wide variation between individuals in the degree of response. In 2004, Van Dongen et al. showed that there are systematic inter-individual differences in neurobehavioural impairment from sleep loss. This study presented evidence of a trait-like differential vulnerability to sleep loss that was replicable and robust to manipulation of sleep history. There has since been widespread investigation of this differential vulnerability to identify predictors. Some of the variation in vulnerability is likely due to genetic factors, and other biological or psychological characteristics, but overall it remains unexplained. The neurocognitive deficits seen in sleep deprived normals and individuals with OSA are similar. Since OSA patients display wide heterogeneity in cognitive impairment relative to disease severity, it may be that this individual variability in symptomatology also has a trait-like basis.
A range of cognitive deficits identified in untreated OSA patients are reflected in patient report of difficulty concentrating, forgetfulness, poor decision making, and falling asleep driving. Of great concern are those symptoms that predispose the patient to increased accident risk, especially when driving. The clinician has a responsibility to both the patient and public to advise of driving risk, particularly in excessively sleepy individuals. Thus assessment of the severity of their chronic daytime sleepiness at initial presentation is crucial. To assist with identification of high risk individuals, a subjective sleepiness tool known as the Epworth Sleepiness Scale (ESS) is widely used in the sleep research and clinical domains. It is broadly applicable since it measures tendency to fall asleep in eight specific activities of normal daily life. However, measurement of sleepiness is difficult, and there are inadequacies in the current conceptual framework for thinking about sleep and wakefulness. Across a 24 hour period, cognitive performance and sleep propensity vary according to: i) the number of hours since the last sleep, ii) the duration of the last sleep, iii) consolidation of the last sleep, and iv) the circadian phase (time of day) at which cognitive performance and sleep propensity is assessed. Sleep and waking function is regulated by the interaction of these processes.

As our knowledge of the control of waking cognitive function has advanced, there has been renewed consideration of how sleep propensity varies with behaviour and the situation in which it is measured. The ESS score (total of eight items) provides a direct estimate of a person’s average level of sleepiness in daily life. Recently, the ESS construct has been considered further to explore the concept...
of somnificity or sleep propensity. The eight items can be grouped according to how soporific (i.e., sleep inducing) the situation is, considering both the subject posture and the environmental circumstances. The somnificity of a situation refers to its capacity to facilitate sleep onset in the majority of people. For example, question 5 (Lying down to rest in the afternoon when circumstances permit) is the most soporific situation and many individuals will report dozing. By contrast, question 8 (In a car, while stopped for a few minutes in traffic) should describe an alerting situation for most people, so any indication of dozing here is a concern. A large study (n = 2,913) in a community sample examining subjective daytime sleepiness found that the distribution of responses on some ESS items was skewed. Most people reported a moderate or high chance of dozing when they lie down to rest, whereas > 85% of subjects reported that they are never likely to doze during conversations or in a car stopped in traffic. The skewed responses in this study support the rationale for the somnificity concept; the sleep propensity of each item in the ESS clearly differs both in situational and behavioural factors.

Since proposed by Johns, this somnificity concept has been explored by researchers to identify individuals at increased risk of dozing in dangerous situations such as driving. Individuals who declare a chance of dozing in a low somnificity situation (i.e., when most people never doze) are vulnerable in safety-critical situations.

Some people are more able to cope with the effects of sleep loss or non-optimally timed sleep opportunities than others. This sleep-loss related vulnerability may reflect stable traits determined by particular genetic
variants. Sleep and wakefulness are regulated by the interaction of homeostatic and circadian (24 hour rhythm) processes. Models recently proposed have included the competing drives of wake and sleep, and situational and trait factors. Normal variation in both processes contributes to differences in sleep phenotypes in aspects of sleep such as duration and timing. Extreme variation can manifest as abnormality or sleep-wake disorders, and the understanding of genetic determinants of some traits and disorders is furthering knowledge of the biological basis of these conditions. Early work in humans has focussed on the circadian rhythm genes since the underlying mechanism has been well-elucidated in animal and Drosophila models. The core set of clock genes involved in the generation of circadian rhythm are known, and Period genes form an integral part of the molecular oscillator of the circadian clock. A molecular feedback loop sets the period of the oscillator, and variations in the genes that control the loop have been related to some individual differences in sleep and circadian phenotypes.

Polymorphisms in human Period genes have been found to be associated with diurnal preference and sleep phase disorders. In 2001 a rare mutation in the PER2 gene was identified as a genetic determinant of abnormal sleep timing in an extended family. Family members suffer from advanced sleep phase disorder resulting in very early sleep timing. An association between a polymorphism in PER3 and delayed sleep phase syndrome was first reported by Ebisawa et al. in 2001. Subsequently Archer et al. recruited subjects with defined diurnal preference and patients with delayed sleep phase syndrome (DSPS) to explore a
possible link with this *PER3* length polymorphism.\textsuperscript{348} They found a robust link between this polymorphism and extreme diurnal preference, including DSPS.\textsuperscript{348} This pivotal finding marked the beginning of a period of research devoted to the exploration of individual resilience or vulnerability to sleep loss mediated by genetic variation. Thus the work of Van Dongen\textsuperscript{23} and Archer has led to a confluence of research interest directed at better understanding the biological and genetic basis of differential vulnerability to sleep loss.\textsuperscript{348}

The primate-specific *PER3* variable-number tandem-repeat (VNTR) polymorphism provides a useful variant to study the impact of clock genes on the regulation and timing of sleep,\textsuperscript{31} and has been intensively studied in relation to sleep-loss related vulnerability (see 4.3.4). Past work has suggested that the *PER3* VNTR polymorphism modulates morning and evening preference, supporting a circadian rhythm function.\textsuperscript{348} To establish the impact of this polymorphism, researchers have used a prospective approach selecting subjects by *PER3* genotype, focussing on homozygotes to enhance variance in vulnerability. In a pivotal study by Viola \textit{et al.}, matched pairs homozygous for the short allele (4-repeat) and long allele (5-repeat) were selected.\textsuperscript{356} The aim of the study was to investigate the functional consequences of this variant for sleep and circadian physiology, plus waking performance using a sleep deprivation protocol. The minor homozygotes (5-repeat) showed a significantly different response to sleep deprivation, with much greater deficits in cognitive function.\textsuperscript{357} This was the first study in humans to provide evidence of some cross-talk between the sleep homeostat and the circadian process.\textsuperscript{353} These genotype-dependent differences
provided support for the hypothesis that individual resilience or vulnerability to
sleep loss is mediated in part by genetic variation. Subsequently there has been
intensive investigation of a proposed resilience or vulnerability to sleep loss
mediated by the \textit{PER3} VNTR polymorphism.\textsuperscript{358} More broadly, further research to
account for trait individual differences in sleep-wake regulation and vulnerability
to sleep loss has been advocated to better manage sleep disorders and fatigue risk
in the workplace.\textsuperscript{24,370}

Individuals with OSA display similar neurocognitive deficits to those in sleep-
deprived normal subjects. Up to 80\% of OSA patients complain of both sleepiness
and cognitive impairments,\textsuperscript{404} and half report personality or mood changes.\textsuperscript{405}
Patients with OSA show variability in the degree of daytime sleepiness and
neurocognitive impairment,\textsuperscript{406} and this is not clearly attributable to severity of
OSA (as measured by AHI), hypoxaemia, or sleep fragmentation.\textsuperscript{19,407,408} Daytime
sleepiness is absent in many OSA patients, yet conversely some with mild disease
and little sleep disruption are very symptomatic.\textsuperscript{409} Normal subjects exposed to
repeated sleep deprivation also show a wide range of trait-like neurocognitive
responses.\textsuperscript{23} The primary domains of neurocognitive dysfunction seen in both OSA
and sleep-deprived normals are: excessive daytime sleepiness, impaired
psychomotor vigilance and executive function, and mood changes.\textsuperscript{20,197,404} Since
there is wide inter-individual variance in the neurobehavioural response to sleep
loss in healthy individuals, perhaps the variability in symptomatology and
neurocognitive deficits seen in OSA patients is trait-like and attributable in part to
genetic factors? Within OSA patients, different patient phenotypes are vulnerable
to neurobehavioural impairment, and identification of these sub-populations would improve clinical management, especially with respect to accident risk.410

In a recent study, I investigated whether driving risk was higher in patients with OSA than in the general community and the nature of the risk (Chapter 3).294 I confirmed that sleep clinic patients with untreated OSA have significantly more crashes than the general population. In addition, I found that there is a strong association between excessive daytime sleepiness and increased report of near-miss crashes supporting the observation that it is those patients with increased sleepiness regardless of OSA severity that are most at risk.294 It will be of great practical benefit to determine biomarkers of individual vulnerability to sleep loss to identify those individuals who most need to avoid accumulating a sleep debt.310

Given the previously reported associations between Period genes (PER2 and PER3) and sleep phenotypes, it is plausible to hypothesis that other Period variants involved in sleep-wake regulation are associated with sleepiness symptoms in subjects with OSA. The purpose of the study reported in this Chapter was to investigate the association of genetic variants (PER2 and PER3) with excessive daytime sleepiness in subjects with OSA. I postulate that the variability in sleepiness symptomatology in OSA cases is modulated by interactions between Period genes, OSA mechanisms (sleep disruption or intermittent hypoxia) and environmental factors. In this analysis, I address the question: Can vulnerability to excessive daytime sleepiness resulting from OSA be predicted on the basis of PER2 or PER3 genotype?
5.4 METHODS

5.4.1 Study design and population

The Western Australian Sleep Health Study (WASHS) is a cohort of sleep clinic patients recruited from the major public adult sleep clinic in Western Australia (WA), and described in detail elsewhere (Section 3.4.1). Briefly, consecutive consenting patients were recruited to the WASHS at first presentation to a sleep physician between 2006 and 2010. All participants provided written informed consent. Approval for the study was granted by the Human Research Ethics Committee at Sir Charles Gairdner Hospital (Trial number: 2004-083).

5.4.1.1 Cases

OSA cases for the current study were drawn from the WASHS cohort. Cases were of predominant Caucasian-European ancestry, had completed a detailed questionnaire capturing patient and family history, had given a blood sample, and were diagnosed with OSA (AHI > 5 events per hour) by overnight PSG.

5.4.1.2 GWAS case sample

Genotype data used in this thesis were generated from a larger study. Previously a genome-wide association study was conducted on WASHS cases (n = 1,301) stratified by body mass index (BMI) into two groups. The low BMI group consisted of individuals with BMI ≤ 30 kg/m², while the high BMI group had BMI ≥ 40 kg/m². Several SNPs from two candidate genes (PER2 and PER3) were selected for their suggested or known involvement in circadian rhythm control and sleep homeostasis by literature review (Section 4.2.4). The PER2 gene is
located on chromosome 2 (base pair position 238,244,038 to 238,288,609) and is 44,572 base pairs long. The \textit{PER3} gene is located on chromosome 1 (base pair position 7,784,703 to 7,845,177) and is 60,475 base pairs long. From the imputed GWAS genotype data I selected 28 \textit{PER2} single-nucleotide polymorphisms (SNPs) and 57 \textit{PER3} SNPs resulting in 1,301 cases with 85 SNPs.

5.4.1.3 GWAS genotyping

Samples from WASHS cases (n = 1,301) were genotyped at the Centre for Applied Genomics, Toronto, Canada on the Illumina HumanOmni2.5-8 BeadChip (Illumina Inc., San Diego, CA, USA) using standard methods.\textsuperscript{411}

5.4.1.4 Hardy-Weinberg Equilibrium

A test for Hardy-Weinberg equilibrium (HWE) was performed for each genotyped SNP prior to imputation (Inclusion criteria: p value $\geq 5.7 \times 10^{-7}$).\textsuperscript{411}

5.4.1.5 Imputation

Using the HapMap 2 CEU (r22.b36) reference set, imputation was performed on the genotyped SNPs using MACH v1.0.16 \textsuperscript{412} and Minimac software.\textsuperscript{413} Only imputed SNPs with the imputation quality measure $R^2 > 0.30$ were included in analyses.

5.4.1.6 Quality control – SNPs

SNPs with call rates (genotyping success rate) of less than 95% and minor allele frequency (MAF) of less than 1% were excluded. After quality control procedures, 1,463,846 SNPs were available for analysis.\textsuperscript{411}
5.4.1.7 Quality control – individuals

Individuals were excluded if: more than 3% of SNP data were missing, reported sex did not match genotyped sex, found to be duplicates, individuals were found to be related using an identity by descent threshold of 0.1875, or individuals were found to have low heterozygosity (> 5 standard deviations below the mean heterozygosity).

5.4.1.8 Linkage Disequilibrium

Prior to analysis, SNPs were tested for evidence of linkage disequilibrium (LD) using the online database SNAP (Section 5.7: Appendix, 5.6.2: Results, Table 5.4 for software link). We calculated LD between each pair of SNPs and systematically removed one of each pair if in perfect LD ([pair-wise $R^2 = 1$ and $D' = 1$], 5.6 Appendix, 5.6.2 Results, Tables 5.4, 5.5a, 5.5b). The genome build LD population was matched to our data; hg18/HapMap Phase ll CEU.

A specific polymorphism in the coding region of the Period3 gene has been extensively investigated in relation to several physiological and pathological traits, including those related to both circadian phase and sleep homeostasis (see Chapter 4, 4.2.4 Period genes). This variable-number tandem-repeat polymorphism (VNTR) consists of a motif encoding 18 amino acids repeated either four ($PER3^4$) or five ($PER3^5$) times. It has been postulated that this $PER3$ VNTR may be important functionally, since the repeat motifs may alter the protein sequence of $PER3$. To determine whether any of the SNPs genotyped or imputed in this study tagged this $PER3$ VNTR polymorphism, two analyses were done.
The *PER3* tag SNP (rs57875989) was uploaded with our complete set of *PER3* SNPs into SNAP (described above) to test for LD. There was no evidence of LD between the *PER3* SNPs in this study and the *PER3* VNTR polymorphism tag SNP. A further analysis was done using the bioinformatics application LocusZoom. Our data file containing *PER3* SNP names and p-values was uploaded via the web form to generate a plot with the *PER3* VNTR (rs57875989) as the reference SNP (Appendix 5.6.1.3, Figure 5.4). There was no usable LD information for the reference SNP in either of the current databases [HapMap Phase II (CEU) or 1,000 Genomes (CEU)]. Thus, the *PER3* VNTR polymorphism is tagged by rs57875989, but this variant has no known LD data in current genomic databases against which LD with our SNPs could be measured.

5.4.1.9 How the case sample data was generated

A merged phenotypic dataset (sleep and questionnaire variables, n = 122) for 1,301 subjects with genetic data was generated. Adjustments to the case sample were made according to missing data, ethnicity, sleep parameters and genotyping failure (Figure 5.1). Cases were excluded if: the ethnicity of both parents was not European, total sleep time was < 150 minutes and AHI < 15 events per hour (i.e. unable to rule in OSA), subjects had a non-OSA primary diagnosis or the BMI was greater than four SDs from the mean (i.e. ≥ 69.5 kg/m²), (Figure 5.1; case sample n = 1,053).
**Figure 5.1.** Flow diagram describing how the case sample was generated.
5.4.2 Techniques

5.4.2.1 Questionnaire data

Smoking and alcohol variables were transformed to calculate standard measures. For smoking, subjects were categorised as never, current or former smokers, with pack years calculated for those who had ever smoked. Responses to alcohol consumption questions were transformed to give standard drinks (10 g alcohol) per week. Caffeine intake was based upon the response to the question “During the past month, have you used coffee, tea or other caffeine drinks to stay awake during your normal waking time?” Possible responses were: never; rarely (has occurred but less than once a week); sometimes (1-2 times per week); frequently (3-4 times per week); always or almost always (5-7 times per week); or don’t know. Usual sleep duration on weekdays and weekends was derived from questionnaire responses for usual time to bed and wake-up time. Epworth Sleepiness Score (ESS) was used as a proxy measure of the degree of daytime sleepiness in cases.88

5.4.2.2 Polysomnography (PSG)

All cases underwent overnight laboratory-based polysomnography (PSG Online 2, Compumedics Ltd, Abbotsford, Australia) and data were analysed according to the 1999 AASM criteria (Profusion 2, Compumedics Ltd, VIC, Australia).85 Sleep was documented using standard electroencephalographic, electro-oculographic and electromyographic criteria.297 AHI and arousal index were defined as the number of events per hour of sleep. Presence of OSA was
5: A NOVEL ASSOCIATION OF POLYMORPHISMS IN THE CIRCADIAN CLOCK PERIOD3 GENE WITH SLEEPINESS IN OBSTRUCTIVE SLEEP APNOEA

defined according to an AHI ≥ 5 events /h. The PSG methodology for WASHS subjects has been described in detail in the cohort profile paper.

5.4.2.3 Statistical methods: General

Linear and logistic regression was used to model the association of genotype and multiple covariates with excessive daytime sleepiness symptomatology in individuals with OSA.

Statistical significance was set at 5% (for non-genetic analyses). Data distribution was reviewed for all variables and cases were described using mean and standard deviations where normally distributed or median and interquartile range (IQR) where skewed. Cases were stratified by sex and differences formally tested using Student’s t-test and Chi-squared test. Pearson’s correlation coefficient was calculated to test the relationship between sleepiness (ESS) and severity of OSA (AHI). Data were analysed using IBM SPSS Statistics (GradPack version 22.0 Release 22.0.0.0, March 11, 2014).

5.4.2.3.1 Outcome variables: ESS

The outcome in these analyses was ESS taken from the WASHS questionnaire. Epworth sleepiness score (ESS) was used as a proxy to define the symptom of excessive daytime sleepiness, an integral part of the clinical syndrome of obstructive sleep apnoea/hypopnoea (OSAHS).

5.4.2.3.2 Somnificity phenotype: Definition

Somnificity refers to specific factors that facilitate sleep onset, such as posture, activity and environment. It has been postulated that ESS can be
transformed into a three-factor structure (reflecting low, medium and high levels of somnificity) to explore the relationship between sleep propensity and other factors. In our study, the three-factor model was used to create a binary variable for somnificity, to classify cases into those who report dozing in a low somnificity situation (i.e. not sleep inducing) versus those who do not. This model allowed exploration of the relationship between genetic factors and the somnificity phenotype in OSA cases. Further detail of the methodology for the three-factor model is described in the appendix, Section 5.6.1.2.

5.4.2.3.3 Epidemiological models

A basic epidemiological model was developed for this within-case analysis. Two models based upon ESS were developed, the outcome variables being ESS continuous and ESS binary [Table 5.1]. Primary exposure variables related to OSA (AHI, arousal index, usual sleep duration, time spent < 90% arterial oxygen saturation, lowest arterial oxygen saturation) were tested individually in the base model (Table 5.2).

Model 1 examined the relationship between ESS as a continuous outcome variable and plausible adjustment and primary exposure variables. Model 2 was a phenotypic model developed to explore ESS binary (somnificity) and dozing risk (Section 5.3.2.3.2 Somnificity phenotype). Cases were categorised into a three-factor structure of ESS to represent low, medium and high somnificity scores. A dichotomous somnificity variable was created to compare cases who report any chance of dozing in a low sleep-inducing situation (e.g. waiting at traffic lights) with those who report no dozing. Low somnificity cases were those who
reported any chance of dozing in either item 6 or 8 of the ESS (Item 6: Sitting and talking to someone; Item 8: In a car, while stopped for a few minutes in traffic). Table 5.2 summarises the two generalised linear models (linear and binary logistic regression) used to model the association of each SNP using an additive genetic model after adjusting for multiple covariates.

Table 5.1. Summary of outcome, general linear model used and number of cases

<table>
<thead>
<tr>
<th>GLM</th>
<th>Outcome</th>
<th>Cases (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: Linear</td>
<td>ESS continuous</td>
<td>1,040</td>
</tr>
<tr>
<td>Model 2: Binary Logistic</td>
<td>Low somnificity</td>
<td>1,036</td>
</tr>
</tbody>
</table>

ESS, Epworth Sleepiness Score.

5.4.2.3.4 Covariates

Models were adjusted for potential confounding variables taken from the WASHS questionnaire (demographic, environmental) and the PSG report (sleep variables, Table 5.2). Demographic variables included sex and age. Obesity is a known risk factor for OSA, and body mass index (BMI [kg/m²]) was therefore included. In addition case selection for the GWAS was defined by BMI and so it was necessary to control for this variable in models (See 5.4.1.2). Environmental exposure variables that may influence sleepiness were measures of sleep fragmentation, hypoxia and sleep duration. Apnoea hypopnoea index (AHI) defined the measure of severity of OSA and presence was defined by AHI ≥ 5 events/h. Arousal index (events/h) represented an alternative measure of sleep fragmentation. Proportion of time spent at an arterial oxygen saturation of < 90% and lowest saturation were the hypoxia measures tested in the models. Usual sleep
duration on weekdays and weekends is another measure of sleep behaviour that can be used to characterise sleep phenotype. Environmental exposures known to be associated with sleep and OSA included caffeine, alcohol and smoking. Caffeine use data was collected from the questionnaire and coded into three categories: never/rarely, sometimes and frequent/always use. Alcohol consumption data from the questionnaire data was transformed to number of standard drinks per week.

**Table 5.2. Summary of variables included in multivariable regression analyses**

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Epworth Sleepiness Score (continuous)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Somnificity (binary)</td>
</tr>
<tr>
<td>Exposure variables</td>
<td>AHI, ARI, proportion of time spent &lt; 90% SaO₂, lowest SaO₂, usual sleep duration</td>
</tr>
<tr>
<td>Adjustment variables</td>
<td>BMI, gender, age, alcohol and caffeine use</td>
</tr>
</tbody>
</table>

Somnificity, sleep propensity (binary) representing no chance of dozing vs any chance of dozing; AHI, apnoea hypopnoea index; ARI, arousal index; SaO₂, arterial oxygen saturation; BMI, body mass index.

5.4.2.3.5 Covariate modelling

Independent predictors of ESS were determined using a stepwise variable selection procedure. Primary exposure and adjustment variables were included if significantly associated at the 20% significance level with each of the dependent variables. Squares of continuous variables and plausible interaction terms were also investigated.

5.4.2.4 Genetic analyses

Genetic association tests were performed using linear regression (ESS continuous) and logistic regression (ESS binary), with each SNP added separately.
to the full base epidemiological model (Table 5.5) and the SNP p-value considered for significance. Primary exposure variables were added separately, one at a time (Table 5.6). Exposure*SNP interactions were also investigated. Multiple testing was corrected using the Bonferroni adjustment, with significance level set to \( p = 0.001 \) (Bonferroni correction: Since 50 SNPs were tested in models, correction was calculated as \( 0.05/50 = 0.001 \)). Analyses for genetic association were performed in the statistical package R version 2.15.2.\(^{416}\)

### 5.5 RESULTS

#### 5.5.1 Participant characteristics

Single-nucleotide polymorphism data were available for 1,301 OSA cases. Exclusions were made according to missing ethnicity, PSG and questionnaire data (only gender known), non-European ancestry, low total sleep time, non-OSA primary diagnosis and an outlying BMI, (Section 5.3.1.9, Figure 5.1). Thus, complete phenotypic and genetic data were available for 1,053 cases.

All cases had OSA diagnosed by laboratory PSG with an AHI threshold of \( \geq 5 \) events per hour. Table 5.3 shows the demographic and PSG characteristics of the cases, stratified by sex. The proportion of cases in the low BMI group (74%) versus the high BMI group (26%) was consistent with the sampling methodology employed for case selection for GWAS outlined in the study population methods (Section 5.4.1.2). There were significantly more men than women (68% vs 32% respectively; \( p < 0.001 \)) in the low BMI group, but significantly more women than men in the high BMI group (58% vs 42% respectively; \( p < 0.001 \)).
Cases were predominantly male, middle-aged and had moderate OSA (AHI 23.8 events per hour [13.5-41.1]). Mean ± SD ESS was 9.8 ± 5.4, and 16% reported moderate to severe excessive sleepiness (ESS ≥ 16). When sleepiness was defined as an ESS > 10, 44.5% of cases with moderate to severe OSA were sleepy. Women were more likely to report dozing in a highly soporific situation (p = 0.03) than men, but not in the low or moderate situations. Age, ESS, lowest arterial oxygen saturation, time spent at an arterial oxygen saturation < 90% and usual duration of sleep did not differ significantly between men and women, but men had significantly higher median scores than women for AHI (27 vs 21 events/hour respectively; p < 0.001) and arousal index (32 vs 29 events/hour respectively; p < 0.001). Women reported significantly more doctor diagnosed depression than men (52% vs 33% respectively; p < 0.001), but there was no significant difference in the proportion of men and women reporting diabetes. Men had a higher weekly alcohol consumption than women (4.5 vs 1.5 standard drinks per week respectively; p < 0.001). There were differences between men and women for smoking (p = 0.04 and 0.02 respectively), but not caffeine consumption (Table 5.3).

5.5.2 Somnificity phenotype

There were 392 cases who reported any chance of dozing in a low somnificity (alerting) situation compared with 661 cases who reported no dozing (case total n = 1,053). Among dozers, 13.5% (53 cases) were not globally sleepy as defined by reporting a total ESS score of < 10. Never-the-less, 7.4% reported that there was a slight chance they would doze “whilst sitting and talking to someone” (item 6), and 3.4% reported a slight chance of dozing “whilst in a car stopped in traffic” (item 8).
Table 5.3. Summary of obstructive sleep apnoea cases

<table>
<thead>
<tr>
<th></th>
<th>All N=1053</th>
<th>Men N=644</th>
<th>Women N=409</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>61.2</td>
<td>51.3</td>
<td>52.4</td>
<td>0.19^</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>51.7 (13.9)*</td>
<td>51.3 (14.2)</td>
<td>52.4 (13.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI, % (n)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI ≤ 30</td>
<td>73.9 (778)</td>
<td>68.0 (529)</td>
<td>32.0 (249)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI ≥ 40</td>
<td>26.1 (275)</td>
<td>41.8 (115)</td>
<td>58.2 (160)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>AHI (events/h)</strong></td>
<td>23.8 (13.5-41.1)*</td>
<td>27.0 (14.9-44.7)</td>
<td>20.7 (11.2-34.6)</td>
<td>&lt;0.001^</td>
</tr>
<tr>
<td><strong>ARI (events/h)</strong></td>
<td>30.9 (21.9-44.4)</td>
<td>32.3 (22.8-46.9)</td>
<td>28.6 (20.3-42.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Lowest SaO2</strong></td>
<td>87 (81-91)</td>
<td>87 (81-90)</td>
<td>87 (81-91)</td>
<td>0.62</td>
</tr>
<tr>
<td><strong>Time &lt;90% SaO2 (mins)</strong></td>
<td>0.6 (0.0-9.3)</td>
<td>0.7 (0.0-8.9)</td>
<td>0.4 (0.0-9.6)</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>ESS (score)</strong></td>
<td>9.8 (5.4)</td>
<td>9.5 (5.3)</td>
<td>10.2 (5.5)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Somnificity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.3 (0.6)</td>
<td>0.3 (0.5)</td>
<td>0.4 (0.6)</td>
<td>0.61</td>
</tr>
<tr>
<td>Medium</td>
<td>1.1 (0.9)</td>
<td>1.1 (0.9)</td>
<td>1.2 (0.9)</td>
<td>0.12</td>
</tr>
<tr>
<td>High</td>
<td>1.9 (0.8)</td>
<td>1.9 (0.8)</td>
<td>2.0 (0.8)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Usual duration sleep (h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weekdays</td>
<td>7.3 (1.9)</td>
<td>7.2 (1.8)</td>
<td>7.4 (2.2)</td>
<td>0.12</td>
</tr>
<tr>
<td>Weekends</td>
<td>7.9 (2.2)</td>
<td>7.9 (2.0)</td>
<td>7.9 (2.4)</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>Depression, % (n)</strong></td>
<td>40.5 (426)</td>
<td>33.2 (214)</td>
<td>51.8 (212)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Diabetes, % (n)</strong></td>
<td>12.3 (129)</td>
<td>11.2 (72)</td>
<td>13.9 (57)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Alcohol (10 g/week)</strong></td>
<td>3.0 (0.0-10.0)</td>
<td>4.5 (1.5-12.0)</td>
<td>1.5 (0.0-4.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Caffeine, % (n)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/rarely</td>
<td>61 (637)</td>
<td>61 (394)</td>
<td>59 (243)</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td>12 (122)</td>
<td>11 (72)</td>
<td>12 (50)</td>
<td>0.15</td>
</tr>
<tr>
<td>Frequent/always</td>
<td>27 (281)</td>
<td>26 (166)</td>
<td>28 (115)</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>44.7</td>
<td>42.5</td>
<td>48.2</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>18.8</td>
<td>17.7</td>
<td>20.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Former</td>
<td>36.5</td>
<td>39.8</td>
<td>31.3</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking, pack years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>27 (13-43)</td>
<td>29 (13-46)</td>
<td>25 (13-34)</td>
<td>0.04</td>
</tr>
<tr>
<td>Former</td>
<td>15 (7-28)</td>
<td>18 (8-29)</td>
<td>12 (5-25)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Note: *Normally distributed continuous data are presented as mean (SD); ^P-value represents t-test of men and women; †Skewed data presented as median [interquartile range (IQR)]; ^P-value represents Mann Whitney U test of men and women; Categorical data presented as % of total (%); P-value represents value from a χ² statistic; BMI (body mass index, kg/m²); AHI, apnoea hypopnoea index; ARI, arousal index; SaO2, arterial oxygen saturation; ESS, Epworth Sleepiness Scale; Depression, self-reported doctor diagnosed depression, Diabetes, self-reported doctor diagnosed diabetes.
5.5.3 Genotypes: Allele frequencies for top SNPs

Genotype and minor allele frequency for the three most strongly associated SNPs are presented in Table 5.4 (full details of the 28 imputed PER2 SNPs and 57 imputed PER3 SNPs are presented in Appendix 5.7). Results: Tables 5.10, 5.10a and 5.10b).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Major homozygote</th>
<th>Heterozygote</th>
<th>Minor homozygote</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>rs697693</td>
<td>GG</td>
<td>60.11</td>
<td>633</td>
</tr>
<tr>
<td>rs7550657</td>
<td>CC</td>
<td>32.86</td>
<td>346</td>
</tr>
<tr>
<td>rs228642</td>
<td>TT</td>
<td>30.77</td>
<td>324</td>
</tr>
</tbody>
</table>

MAF*, minor allele frequency (calculated directly from imputed doses, See Appendix, Results 5.6.2, Tables 5.6a and b for full details); Genotype, imputed dose converted to genotype for the purpose of this table. When imputed dose value was neither 0, 1 or 2, the value was converted to the most probable value by rounding, See Section 5.3.1.5 for imputation methodology.

One PER2 SNP – rs4429421 – had a minor allele frequency of < 1% (and R² value for imputation was < 0.3) and was excluded from subsequent analysis.

Overall quality of imputation was high with an imputation value > 0.90 for 92% of SNPs. Imputation value was acceptable for the remainder (n = 6) with 0.3 ≤ R² ≤ 0.90 (See 5.7 Appendix; 5.7.2 Results, supplementary tables 5.10, 5.10a and 5.10b). Linkage disequilibrium (LD) analysis found seven PER2 SNPs and twenty eight PER3 SNPs were in perfect LD and therefore pruned prior to analysis to reduce the multiple testing issue (Section 5.3.2.4 Methods). Therefore, of 85 SNPs available for analysis, 35 were removed leaving 50 SNPs in the current study.
5.5.4 Covariate modelling

The final multivariate non-genetic models are presented in Table 5.5. Multivariate modelling indicated that ESS was associated with age \( (p = 0.05) \), BMI \( (p < 0.001) \) and caffeine use \( (p < 0.001) \). With increasing age, obesity and caffeine use, subjects had an increased risk of an elevated ESS. Model 2 showed that dozing in a low somnificity situation was associated with BMI \( (p < 0.001) \), caffeine use \( (p < 0.001) \) and alcohol use \( (p = 0.001) \), but not age \( (p = 0.518) \) or sex \( (p = 0.055) \). Cases (those who reported any chance of dozing) had increased risk of dozing if obese and with increased report of caffeine use. Higher alcohol use was associated with less chance of reported dozing, but the effect size was small. Women were less likely to report dozing than men, suggesting a possible gender effect, but this effect did not reach statistical significance \( (p = 0.055) \). Smoking was tested but was not significant in univariable models. Smoking was retested in the final multivariable model but remained non-significant. The same base epidemiological model was applied to both outcome variables to support consistent interpretation of data.
Table 5.5. Base epidemiological model for two outcome variables of Epworth Sleepiness Score

<table>
<thead>
<tr>
<th>Model</th>
<th>Model 1: ESS continuous N=1040</th>
<th>Model 2: ESS binary* N=1036</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>β</td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td>0.024</td>
<td>(0.000, 0.048)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.015</td>
<td>(-0.665, 0.694)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI≤30</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>BMI≥40</td>
<td>2.079</td>
<td>(1.335, 2.823)</td>
</tr>
<tr>
<td>Caffeine use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/rarely</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td>1.898</td>
<td>(0.888, 2.908)</td>
</tr>
<tr>
<td>Frequent/always</td>
<td>2.217</td>
<td>(1.455, 2.980)</td>
</tr>
<tr>
<td>Alcohol, std drinks/wk</td>
<td>-0.019</td>
<td>(-0.051, 0.014)</td>
</tr>
</tbody>
</table>

BMI, body mass index; ESS, Epworth sleepiness score; β, beta coefficient; CI, confidence interval; L, lower; U, upper; OR, odds ratio; *ESS binary, representing somnificity or sleep propensity, cases reported any chance of dozing vs no chance of dozing in a low somnificity situation.

5.5.5 Primary exposure variables

Primary exposure variables of sleep disruption, hypoxia and sleep duration were tested in the base epidemiological models for association with the outcome of sleepiness (Table 5.6). Apnoea hypopnoea index (AHI) was associated with the ESS outcome in both models, adjusting for age, sex, BMI, caffeine and alcohol use. As AHI increased, the odds of reporting more sleepiness (p <0.001) or dozing (p = 0.056) increased. This relationship was consistent across models, although not statistically significant for somnificity (p = 0.056). Although there was a significant correlation between AHI and ESS (r = 0.18, 95% CI: 0.12, 0.24, p < 0.001), very little of the sleepiness was explained by AHI (r² = 0.032). A box plot illustrating ESS
for cases of mild, moderate and severe OSA shows that the distribution is similar for each severity group (Figure 5.3).

**Figure 5.3.** Box plot comparing ESS for mild, moderate and severe OSA cases.

Both hypoxia variables (lowest arterial oxygen saturation and time spent < 90% arterial oxygen saturation) were significant in the models (Table 5.6). For lowest arterial oxygen saturation there was an inverse relationship with the outcome (p < 0.001) whereby, as less time was spent at low oxygen saturations an individual was less likely to report excessive sleepiness or dozing. Similarly, greater time spent at < 90% arterial oxygen saturation was associated (p = 0.001) with an increased risk of reporting excessive daytime sleepiness or dozing. The sleep duration variables were not associated with ESS and were not considered further.
Table 5.6. Primary exposure variables tested for association in base epidemiological models

<table>
<thead>
<tr>
<th>Model</th>
<th>β</th>
<th>95% CI</th>
<th>p value</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep disruption*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• AHI, events/h</td>
<td>0.028</td>
<td>(0.014, 0.041)</td>
<td>&lt;0.001</td>
<td>1.005</td>
<td>(1.000, 1.011)</td>
<td>0.056</td>
</tr>
<tr>
<td>• ARI, events/h</td>
<td>0.025</td>
<td>(0.009, 0.041)</td>
<td>0.002</td>
<td>1.004</td>
<td>(0.997, 1.011)</td>
<td>0.232</td>
</tr>
<tr>
<td>Hypoxia#</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Lowest SaO₂</td>
<td>-0.086</td>
<td>(-0.123, -0.048)</td>
<td>&lt;0.001</td>
<td>0.974</td>
<td>(0.958, 0.989)</td>
<td>0.001</td>
</tr>
<tr>
<td>• Time spent &lt; 90%, mins</td>
<td>0.018</td>
<td>(0.009, 0.027)</td>
<td>&lt;0.001</td>
<td>1.005</td>
<td>(1.001, 1.009)</td>
<td>0.012</td>
</tr>
<tr>
<td>Sleep duration^</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Total weekdays, h</td>
<td>0.140</td>
<td>(-0.009, 0.290)</td>
<td>0.065</td>
<td>0.949</td>
<td>(0.886, 1.017)</td>
<td>0.138</td>
</tr>
<tr>
<td>• Total weekends, h</td>
<td>0.111</td>
<td>(-0.056, 0.278)</td>
<td>0.192</td>
<td>0.988</td>
<td>(0.922, 1.051)</td>
<td>0.711</td>
</tr>
</tbody>
</table>

ESS, Epworth sleepiness score; *ESS binary, representing somnificity or sleep propensity, cases reported any chance of dozing vs no chance of dozing in a low somnificity situation; ß, beta coefficient; CI, confidence interval; L, lower; U, upper; AHI, apnoea hypopnoea index; ARI, arousal index; SaO₂, arterial oxygen saturation; Time spent<90%, time spent at an arterial oxygen saturation of <90%; Somnificity, sleep propensity (binary) representing no chance of dozing vs any chance of dozing.

* Model adjusted for age, sex, BMI, caffeine and alcohol + sleep disruption variable
# Model adjusted for age, sex, BMI, caffeine and alcohol + hypoxia variable
^ Model adjusted for age, sex, BMI, caffeine and alcohol + sleep duration variable

5.5.6 Genetic associations

5.5.6.1 Single-nucleotide polymorphisms associated with Epworth Sleepiness Score

There were no associations between SNPs and ESS in multivariate analyses when the correction for multiple testing (Bonferroni) was taken into consideration. However, five SNPs were associated with ESS after adjusting for AHI and a SNP*AHI interaction (Table 5.7). SNP rs697693 (PER3) showed the strongest association (SNP ß -1.042, 95%CI: -1.854, -0.230, p = 0.012; SNP*Int ß 0.036, 95%CI: 0.017, 0.055, p = 1.7 x 10⁻⁴). SNP rs11121030 (PER3) and SNP rs228642
(PER3) showed the next strongest associations of a similar magnitude (Table 5.7). No associations remained significant after adjustment for multiple testing (P threshold = 0.001). Multivariate results for ESS and these SNPs are reported in Table 5.7 (Supplemental Table 5.12). There were no significant associations between PER2 SNPs and ESS in multivariate analyses (Supplemental Table 5.11).
### Table 5.7. Results of multivariate analyses for Epworth Sleepiness Score (ESS) and the three PER3 single-nucleotide polymorphisms (SNPs) with the strongest association

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.024</td>
<td>(0.000, 0.048)</td>
<td>0.051</td>
</tr>
<tr>
<td>Sex</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.015</td>
<td>(-0.665, 0.694)</td>
<td>0.967</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI≤30</td>
<td>2.079</td>
<td>(1.335, 2.823)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI≥40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine use</td>
<td>1.898</td>
<td>(0.888, 2.908)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Never/rarely</td>
<td>2.217</td>
<td>(1.455, 2.980)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sometimes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequent/always</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol, std drinks/wk</td>
<td>-0.019</td>
<td>(-0.051, 0.014)</td>
<td>0.261</td>
</tr>
<tr>
<td>BMI, body mass index; OR, odds ratio;*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SNP rs697693</strong>*</td>
<td>0.165</td>
<td>(-0.375, 0.706)</td>
<td>0.548</td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>0.136</td>
<td>(-0.400, 0.673)</td>
<td>0.619</td>
</tr>
<tr>
<td><strong>SNP rs697693^</strong></td>
<td>-1.042</td>
<td>(-1.854, -0.230)</td>
<td>0.012</td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>-0.028</td>
<td>(-0.060, 0.004)</td>
<td>0.085</td>
</tr>
<tr>
<td>+ SNP*AHI interaction</td>
<td>0.036</td>
<td>(0.017, 0.055)</td>
<td>1.7 x 10^-4</td>
</tr>
<tr>
<td><strong>SNP rs11121030</strong>*</td>
<td>-0.301</td>
<td>(-1.385, 0.783)</td>
<td>0.586</td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>-0.311</td>
<td>(-1.388, 0.765)</td>
<td>0.549</td>
</tr>
<tr>
<td><strong>SNP rs11121030^</strong></td>
<td>-2.720</td>
<td>(-4.399, -1.042)</td>
<td>0.002</td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>-0.125</td>
<td>(-0.134, -0.117)</td>
<td>0.003</td>
</tr>
<tr>
<td>+ SNP*AHI interaction</td>
<td>0.080</td>
<td>(0.037, 0.123)</td>
<td>2.7 x 10^-4</td>
</tr>
<tr>
<td><strong>SNP rs228642</strong>*</td>
<td>0.124</td>
<td>(-0.323, 0.571)</td>
<td>0.586</td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>0.131</td>
<td>(-0.312, 0.575)</td>
<td>0.562</td>
</tr>
<tr>
<td><strong>SNP rs228642^</strong></td>
<td>-0.800</td>
<td>(-1.480, -0.119)</td>
<td>0.021</td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>-0.002</td>
<td>(-0.023, 0.019)</td>
<td>0.854</td>
</tr>
<tr>
<td>+ SNP*AHI interaction</td>
<td>0.028</td>
<td>(0.012, 0.044)</td>
<td>4.5 x 10^-4</td>
</tr>
</tbody>
</table>

**BMI, body mass index; OR, odds ratio;**
* Model adjusted for age, sex, BMI, caffeine and alcohol;
# Base epidemiological model + SNP + AHI;
^ Base epidemiological model + SNP + AHI + SNP*AHI interaction.
5.5.6.2 Single nucleotide polymorphisms associated with ESS binary

Three SNPs were associated with ESS binary (somnificity) \([p = 0.001]\) in multivariate analyses, after adjusting for AHI and a SNP*AHI interaction (Table 5.8). SNP rs697693 \((PER3)\) showed the strongest association (SNP OR 0.560, 95%CI: 0.393, 0.797, \(P = 0.001\); SNP*Int OR 1.021, 95%CI: 1.011, 1.030, \(P = 9.3 \times 10^{-6}\)). An interaction plot for rs697693 explores the relationship between somnificity, severity of OSA and genotype (Figure 5.4). Severity of OSA (represented by mean AHI) is higher in dozers \((36.1 \pm 30.6, \text{range 5.0-156.9})\) than non-dozers \((30.3 \pm 24.8, \text{range 5.1-152.4})\), however the association of AHI with likelihood of dozing appears dependent upon genotype. Minor homozygotes \((AA)\) are at risk of dozing at a lower AHI value \((24 \text{ events per hour})\), relative to major homozygotes \((GG)\) who are most at risk when OSA is more severe \((40 \text{ events per hour})\). For heterozygotes \((AG)\), the risk of dozing is equivalent at the same level of AHI \((30 \text{ events per hour})\) see Figure 5.3). The bar plot in Figure 5.4 illustrates the genotype-dependent differences relative to severity of OSA in dozers versus non-dozers.

SNP rs7550657 \((PER3)\) and SNP rs228642 \((PER3)\) showed the next strongest associations of a similar magnitude (Table 5.8). Multivariate results for somnificity and the three top SNPs are reported in Table 5.8 (Supplemental Tables 5.14 and 5.16). There were no significant associations between \(PER2\) SNPs and somnificity in multivariate analyses (Supplemental Tables 5.13 and 5.15).
Table 5.8. Results of multivariate analyses for ESS binary and the three PER3 single-nucleotide polymorphisms (SNPs) with the strongest association

<table>
<thead>
<tr>
<th>Epidemiological model</th>
<th>ESS binary* N=1036</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td>1.003</td>
<td>(0.993, 1.013)</td>
<td>0.518</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>0.759</td>
<td>(0.572, 1.006)</td>
<td>0.055</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>Male</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI≤30</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI≥40</td>
<td>1.721</td>
<td>(1.273, 2.326)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caffeine use</td>
<td>Male</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI≤30</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI≥40</td>
<td>1.721</td>
<td>(1.273, 2.326)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol, std drinks/wk</td>
<td>Never/rarely</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sometimes</td>
<td>2.151</td>
<td>(1.436, 3.220)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Frequent/always</td>
<td>2.174</td>
<td>(1.595, 2.963)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SNP rs697693*</td>
<td>1.081</td>
<td>(0.865, 1.351)</td>
<td>0.494</td>
<td></td>
</tr>
<tr>
<td>SNP rs697693#</td>
<td>1.075</td>
<td>(0.860, 1.345)</td>
<td>0.525</td>
<td></td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>1.005</td>
<td>(1.000, 1.011)</td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td>SNP rs697693^</td>
<td>0.560</td>
<td>(0.393, 0.797)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>0.974</td>
<td>(0.959, 0.989)</td>
<td>9.1 x 10^-4</td>
<td></td>
</tr>
<tr>
<td>+ SNP*AHI interaction</td>
<td>1.021</td>
<td>(1.011, 1.03)</td>
<td>9.3 x 10^-6</td>
<td></td>
</tr>
<tr>
<td>SNP rs7550657*</td>
<td>1.032</td>
<td>(0.858, 1.241)</td>
<td>0.740</td>
<td></td>
</tr>
<tr>
<td>SNP rs7550657#</td>
<td>1.031</td>
<td>(0.857, 1.241)</td>
<td>0.744</td>
<td></td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>1.005</td>
<td>(1.000, 1.011)</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td>SNP rs7550657^</td>
<td>0.602</td>
<td>(0.447, 0.811)</td>
<td>8.0 x 10^-4</td>
<td></td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>0.988</td>
<td>(0.978, 0.998)</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>+ SNP*AHI interaction</td>
<td>1.016</td>
<td>(1.009, 1.024)</td>
<td>9.8 x 10^-6</td>
<td></td>
</tr>
<tr>
<td>SNP rs228642*</td>
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<td>(0.870, 1.257)</td>
<td>0.630</td>
<td></td>
</tr>
<tr>
<td>SNP rs228642#</td>
<td>1.048</td>
<td>(0.872, 1.260)</td>
<td>0.617</td>
<td></td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>1.005</td>
<td>(1.000, 1.011)</td>
<td>0.056</td>
<td></td>
</tr>
<tr>
<td>SNP rs228642^</td>
<td>0.611</td>
<td>(0.454, 0.824)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>+ AHI, events/h</td>
<td>0.989</td>
<td>(0.979, 0.998)</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>+ SNP*AHI interaction</td>
<td>1.016</td>
<td>(1.009, 1.024)</td>
<td>1.1 x 10^-5</td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index; OR, odds ratio; *ESS binary, somnificity or sleep propensity representing any chance of dozing vs no chance of dozing in a low somnificity situation; # Model adjusted for age, sex, BMI, caffeine and alcohol; ^ Base epidemiological model + SNP + AHI; * Model adjusted for age, sex, BMI, caffeine and alcohol; # Base epidemiological model + SNP + AHI; ^ Base epidemiological model + SNP + AHI + SNP*AHI interaction.
Figure 5.3. Association between severity of OSA (mean AHI) and risk of dozing in an alerting situation by PER3 genotype (rs697693).
Figure 5.4. Association of rs697693 with mean AHI according to genotype, for dozers versus non-dozers. The numbers of cases having each genotype are noted in each bar. The error bars represent the standard error for the means.
5.6 Discussion

This is the first study to investigate whether Period gene variants known to be associated with control of circadian rhythm are associated with the excessive sleepiness symptom in OSA. The current study demonstrates a novel association between three \textit{PER3} SNPs and increased risk of dozing in a low somnificity (alerting) situation. The relationship was mediated via an interaction between \textit{PER3} genotype and severity of OSA (AHI index), a surrogate measure of sleep disruption. From these results it might be inferred that \textit{PER3} genotype variation contributes to increased risk of dozing or sleepiness symptoms in OSA patients. This may account in part for the known poor correlation between disease severity and sleepiness symptomatology in OSA.\textsuperscript{149}

The present study is the first to report an association between \textit{PER3} variants and subjective sleepiness in a well-phenotyped cohort of OSA patients. Previous candidate gene and linkage studies have reported associations between Period genetic variants and chronotype\textsuperscript{360,417} and both advanced\textsuperscript{418} and delayed sleep phase disorders.\textsuperscript{348,355} A large body of work explored the finding by Viola \textit{et al.} that a \textit{PER3} VNTR polymorphism predicts both sleep structure and waking performance in healthy individuals.\textsuperscript{356} In 2007 Gottlieb \textit{et al.} conducted the first GWAS for sleep phenotypes in the Framingham cohort and identified a locus on chromosome 5 with genome-wide association to sleepiness (as measured by ESS), but this finding has not been replicated.\textsuperscript{25} My study extends previous observations as an association between \textit{PER3} variants and the disease-related trait of sleepiness in OSA has not previously been reported.
A strength of the current study is its design. This within-case analysis of a large, well-phenotyped cohort of OSA cases has used the most powerful study design for the detection of interaction effects. We developed a clear primary hypothesis for our study based upon the biological plausibility of a role for PER2 and PER3 genes in the expression of sleepiness phenotypes. This was based upon several relevant prior findings of association between PER2 and PER3 variants and sleep-wake regulation with evidence from both animal models and humans.330 As with all genetic association studies, this study has the potential limitations of use of self-reported variables (e.g. ESS) and a modest sample size. Although the sample is large by comparison with other association studies of sleep phenotypes, the study was likely underpowered to detect very modest genetic effects and/or interactions. A further limitation is that not all previously reported variants were selected for genotyping. However, attention paid to correct genotyping quality control methods was supported by low error rates, and conservative multiple testing corrections (Bonferroni) were applied.317 The primary limitation of the study is the difficulty of interpreting the biology that underlies the sleepiness trait in OSA due to its biological and phenotypic complexity.317 However, the comprehensive phenotyping of OSA cases strengthens our findings and the results of this study are hypothesis generating and warrant further investigation.

Our findings suggest an interaction effect between PER3 genotype and risk of dozing in individuals, mediated by severity of OSA. This finding may provide a partial explanation for the weak relationship between OSA severity and sleepiness symptoms. Some large samples show a statistically significant correlation between
AHI and daytime sleepiness (ESS) but this rarely exceeds $r = 0.4$. In the current study, there was a significant correlation between AHI and ESS ($r = 0.18$, 95% CI: 0.12, 0.24, $p < 0.001$), but very little of the sleepiness was explained by AHI ($r^2 = 0.032$). Other primary exposure variables (arousal index, hypoxia and sleep duration) were investigated but no associations were found (Table 5.6). The mechanisms postulated to cause the excessive sleepiness in OSA are intermittent hypoxia, sleep fragmentation, or both, but the relative contributions of each to daytime sleepiness and neurobehavioural impairment remain unclear. A recent review by Reynolds and Banks concluded that overall, evidence from sleep fragmentation studies confirmed an association with objective and subjective sleepiness. Intermittent hypoxia is common in OSA but varies widely in degree, and can lead to cognitive dysfunction including sleepiness. Clinical and experimental evidence suggests that exposure to prolonged periods of intermittent hypoxia may play a critical role in the development of neurocognitive deficits of sleepiness, mood disturbance and impaired cognition. Colt et al. conducted an experiment in 1991 to differentiate the effects of sleep fragmentation from intermittent hypoxia in producing the sleepiness associated with OSA. They treated OSA with continuous positive airway pressure and introduced periods of desaturation experimentally, but sleepiness did not recur with this intermittent hypoxia alone. Thus sleep fragmentation and intermittent hypoxia probably play a combined role, but my results suggest that sleep disruption contributes to the risk of dozing more so than hypoxia.
Over the past fifteen years a developing field of research has addressed differing neurobehavioural vulnerability to sleep loss in humans. Sleep deprivation studies in healthy normals found that neurobehavioural deficits from sleep loss varied considerably between individuals, but were stable within individuals. It has been postulated that this variance (up to 92%) may be due to trait-like differential vulnerability to impairment from sleep loss. There is some evidence that common genetic variants involved in sleep-wake, circadian and cognitive regulation may underlie some of the variation in vulnerability to sleep loss. In a review of daytime sleepiness Cluydts et al. suggested that these ‘trait’ or person-specific aspects of sleepiness have been largely ignored in sleep-wake research in the past. However rapid evolution of genetic analysis techniques has led to report of several associations between genetic variants and sleep disorders and traits of potential relevance to defining the components of an individual sleep phenotype.

Despite extensive research directed at the concept of sleepiness, its measurement and its consequences for society, it remains challenging to separate circadian and homeostatic influences and to assess inter-individual variability. The two-process model of sleepiness predicts important fluctuations in the sleepiness state across 24 hours, but does not take into account an individual’s stable characteristic level of sleepiness or ‘trait’ level. A behavioural definition of sleepiness is the subject’s tendency to doze off or fall asleep, i.e. sleep propensity. Thus sleepiness reflects a physiological need for sleep just as hunger reflects a need for food. How much sleep do we need? A review by Ferrara and De Gennaro
concluded that the sleep phenotype for an individual needs to take into account age, gender, short or long sleep requirement (duration), morning/evening typology (chronotype) and sleep propensity across the sleepy-alert dimension. Thus many components of an individual sleepiness trait are endogenous. Twin studies have shown 38% heritability for sleepiness. Examples of heritable factors that contribute to the sleepiness phenotype are; sleep duration (i.e. individual sleep need), sleep timing (morningness/eveningness) and sleep structure (i.e. sleep stage proportions). Thus, the large and often unexplored individual differences that exist among individuals in their need for sleep are likely underpinned by genetic determinants for each sleep phenotype component.

Previous studies have explored associations with circadian clock genes since disturbances in circadian rhythms are prominent in mood disorders, and both low mood and depression are highly prevalent in OSA patients. Two of the PER3 variants of interest in our study have been associated with other sleep and non-sleep phenotypes. Disruption in circadian gene expression is associated with increased incidence of some cancers. A recent study explored the relationship between common genetic variation in circadian rhythm genes and risk of epithelial ovarian cancer (EOC). An association between the PER3 variant rs697693 and risk of overall EOC was reported, but did not survive multiple testing. Nievergelt et al. reported suggestive evidence for the association of the circadian gene PER3 (including the specific variant rs228642) with bipolar disorder. Finally, Antypa et al. provided preliminary evidence that the rs228642 SNP in PER3 is associated with disruption in sleep pattern after the experience of stress. When combined,
these study results suggest that disruptions of normal circadian biology have the potential to influence a range of disease-related pathways and sleep phenotypes.

My study has found an association between \textit{PER3} variants and a sleepiness phenotype in OSA patients. Cases who were homozygous for the minor allele (AA) of rs697693 (5.3\%, Table 5.4) are at risk of dozing for less severe OSA (a lower AHI of 24) than either major homozygotes (GG) or heterozygotes (AG) [Figure 5.6]. Thus it may be speculated that the dominant allele (G) confers a degree of resilience to the sleep disruption of OSA in major homozygotes and heterozygotes, while minor homozygotes are vulnerable. Viola \textit{et al.} found genotype-dependent differences between healthy subjects in their response to sleep loss supporting the concept of individual resilience or vulnerability mediated in part by genetic variation.\textsuperscript{356} Research exploring differential neurobehavioural vulnerability to sleep loss in healthy subjects\textsuperscript{24} has direct relevance to understanding the heterogeneity of cognitive and performance deficits seen in patients with OSA.\textsuperscript{404} Both the sleepiness symptom and the OSA phenotype are complex conditions. As such, the expression of excessive sleepiness due to OSA will likely be comprised of many genetic variants of small effect, lending support to our finding of three \textit{PER3} variants associated with risk of dozing, a marker of sleepiness.

The sleepiness phenotype for a subject with OSA will be defined by their trait level of sleepiness in addition to the ‘load’ imposed by sleep disruption. The sleep disruption of OSA imposes a neurobiological ‘load’ which varies according to disease severity, (i.e. with respect to both the frequency of breathing pauses and degree of hypoxaemia). Clinical sleep researchers have begun to explore the
phenotypic variability in neurocognitive deficits seen in patients with OSA. Two studies have examined subjects with untreated OSA challenged with further sleep deprivation. Wong et al. compared OSA subjects with controls using a 40 hour total sleep deprivation protocol. They found no significant differences between OSA cases and controls, with the same decrements in cognitive function across 24 hours (i.e. worst performance in the early morning and decline in performance with increasing time awake). However there was significant individual variability in vulnerability as measured by performance decrements in sustained attention, simulated driving and subjective sleepiness. Vakulin et al. conducted a study to systematically examine the phenotypic variability in simulated driving performance in OSA subjects. They exposed OSA patients to sleep restriction and low dose alcohol then conducted simulated driving and other cognitive tests to search for predictors of increased driving risk among individuals. Some cases (40%) consistently and rapidly developed sleepiness and cognitive impairment during restricted sleep, while others (60%) showed trait-like resistance (similar to controls). No baseline OSA-related measures predicted resilient or vulnerable drivers. However there was wide heterogeneity between OSA subjects in driving simulator performance. Both these studies of OSA cases identified wide heterogeneity between individuals in their vulnerability to sleep deprivation similar to that seen in studies of healthy controls, and pointing to trait differences. Their findings are consistent with the notion that genotype influences neurobehavioural response to sleep disruption, but neither study design allows speculation as to the genetic determinants of this variability. Well-designed genetic association studies of subjects with OSA with and without sleepiness, drawn from
the same population are needed to help explain the factors that underlie individual differences in vulnerability.\textsuperscript{36}

5.6.1 Clinical Relevance

If replicated, the clinical relevance of my study findings would be that these \textit{PER3} variants could serve as biomarkers for individuals who are more vulnerable to sleep loss, and therefore predisposed to excessive daytime sleepiness and increased accident risk. Indeed, when the somnificity construct is considered, \(13.5\%\) (\(n = 53\)) of low somnificity cases (i.e. those that dozed in an alerting situation) were not clinically sleepy as defined by reporting a total ESS score of less than 10. Thus, these cases would not be identified at first presentation as being excessively sleepy (ESS \(\geq 16\)), even though they report a slight chance of dozing in a potentially hazardous situation (i.e. whilst in a car stopped in traffic). Knowledge of both the somnificity score and presence of biomarkers that signal vulnerability to sleep loss would facilitate clinical judgement by the physician at initial presentation with respect to driving and safety-critical tasks.

5.6.2 Conclusion

In summary, the present study investigated potential associations between excessive sleepiness (risk of dozing in an alerting situation), OSA-related phenotypic variables and genotypic variants. Two primary exposure variables (AHI and hypoxia) were significantly associated with sleepiness (ESS), a finding that supports current understanding of the mechanisms purported to underlie excessive sleepiness in OSA. In addition, three SNPs were found to be associated with risk of dozing, with genotype-dependent differences mediated by severity of
OSA as indicated by AHI, but not hypoxia. These associations between \textit{PER3} variants and risk of dozing in OSA cases suggest a potential role for \textit{PER3} variants as biomarkers of vulnerability to sleep disruption.

5.6.3 \textbf{Future research}

The three \textit{PER3} variants found to have an association with risk of dozing in OSA patients warrant replication in other well-phenotyped OSA cohorts. Substantial knowledge regarding genotype-dependent vulnerability to sleep loss has been gained using a prospective approach whereby subjects are recruited according to genotype. The genotype frequencies of the top three SNPs we identified would allow such a study design. If OSA subjects could be recruited on the basis of genotype, studies of cognitive performance in response to sleep deprivation may elucidate the role of genetic variants interacting with OSA-related variables to promote excessive sleepiness symptoms. Determination of genetic biomarkers of individual differences in vulnerability to sleep loss would help identify those people most in need of prompt diagnosis and treatment of sleep disorders. This is of particular relevance to clinical management of cases in safety-critical occupations who are most exposed to accident risk.
5.7 APPENDIX

5.7.1 Methods

Following is a more detailed explanation of the concept of somnificity as applied in the association analysis between \textit{PER2} and \textit{PER3} variants, and risk of dozing in an alerting situation.

5.7.1.1 Somnificity: The three-factor model

A three-level sub-scale structure of the ESS was proposed by Olaithe et al to better reflect facets of sleepiness encompassing the broad features of a person's posture, activity and environment that facilitate sleep onset for a majority of people, the majority of the time.\textsuperscript{174} Simply, the eight items of the ESS score can be grouped to reflect situations of low, moderate and high somnificity (Figure 5.6). For a description of the single-factor structure of ESS which represents average sleep propensity in daily situations, see Section 2.5.3.2. The three-factor structure was created as follows; high somnificity combined items 1, 2 and 5, moderate somnificity combined items 3, 4 and 7, and low somnificity combined items 6 and 8 (Figure 5.3).\textsuperscript{176}
The single-factor structure of ESS gives a total score which reflects global sleepiness. Where individuals do not achieve a clinically sleepy total score (ESS \( \geq 10 \)), they may concede to falling asleep under conditions that do not normally induce sleepiness (ie. a positive response to Items 6 and 8 of the ESS). This was shown to be the case in the clinical sample of the Olaithe study where subjects who were not sleepy (ESS < 10) reported a slight chance of dozing in items 6 and 8. Mean subscale scores for low (mean±SD, 0.3±0.6), medium (mean±SD, 1.1±0.9) and high (mean±SD, 1.9±0.8) somnificity (maximum 3) in our cohort were not significantly different from values reported by Olaithe et al.
(n = 693 cases) in a similar cohort (Table 5.9), supporting internal consistency of the somnificity construct between these OSA cohorts.

Table 5.9. ESS total score and subscale scores somnificity constructs for community sample, clinical sample (Olaite 2013) and WASHS cohort.

<table>
<thead>
<tr>
<th>Scale total M±SD</th>
<th>Community Sample n=356</th>
<th>Clinical Sample n=693</th>
<th>WASHS Cohort N=1053</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESS total score</td>
<td>7.2 ± 4.2</td>
<td>10.0 ± 5.3</td>
<td>9.8 ± 5.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Low somnificity score</td>
<td>0.1 ± 0.3</td>
<td>0.3 ± 0.6</td>
<td>0.3 ± 0.6</td>
<td>0.001</td>
</tr>
<tr>
<td>(max. 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium somnificity score</td>
<td>0.8 ± 0.7</td>
<td>1.2 ± 0.9</td>
<td>1.1 ± 0.9</td>
<td>0.001</td>
</tr>
<tr>
<td>(max. 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High somnificity score</td>
<td>1.5 ± 0.7</td>
<td>1.9 ± 0.8</td>
<td>1.9 ± 0.8</td>
<td>0.724</td>
</tr>
<tr>
<td>(max. 3)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

ESS, Epworth Sleepiness Scale; WASHS, West Australian Sleep Health Cohort.

Thus if the construct scores were available, a high score on the low somnificity scale could raise concern about sleepiness since the individual may fall asleep under potentially hazardous circumstances. In this study, somnificity was dichotomised to group cases who reported any chance of dozing in a low somnificity situation versus those who do not. The underlying aim of this model is to identify those cases who report dozing in a low somnificity situation, irrespective of their total ESS score. In the context of driving and safety critical environments, identification of these individuals is crucial.
5.7.1.2 Linkage Disequilibrium

The graph below is a regional plot generated in a bioinformatics program named LocusZoom to assess whether the PER3 tag SNP (rs57875989) was in linkage disequilibrium with any of the SNPs in our study.414

![Regional plot of Period3 gene (n=30 SNPs) on chromosome 1. There was no usable LD information for the reference SNP (rs57875989; PER3 VNTR), so no r^2 values are reported (see Figure 5.4 below for comparison). The genome build LD population was matched to our data; hg18/HapMap Phase II CEU.](image)

**Figure 5.6.** Regional plot of Period3 gene (n=30 SNPs) on chromosome 1. There was no usable LD information for the reference SNP (rs57875989; PER3 VNTR), so no r^2 values are reported (see Figure 5.4 below for comparison). The genome build LD population was matched to our data; hg18/HapMap Phase II CEU.
The graph below is a regional plot of the PER3 SNPs genotyped for this study showing linkage disequilibrium (LD) values relative to the top SNP (rs697693). Prior to analysis, all SNPs were tested for evidence of LD pairwise, removing one of each pair if in perfect LD (Section 5.3.1.8). The results in the plot confirm that none of the remaining SNPs were in LD.

**Figure 5.7.** Regional plot of Period3 gene on chromosome 1 showing linkage disequilibrium (LD; \( r^2 \) value) for PER3 single-nucleotide polymorphisms (SNPs; n=30)) analysed in this study. Pairwise LD is calculated for each SNP relative to the top SNP (rs697693). The genome build LD population was matched to our data; hg18/HapMap Phase II CEU.
5.7.2 Results

The results below in Tables 5.10, 5.10a and 5.10b present full details of the 28 imputed \textit{PER2} SNPs and 57 imputed \textit{PER3} SNPs in this analysis. Linkage disequilibrium (LD) analysis found seven \textit{PER2} SNPs and twenty eight \textit{PER3} SNPs were in perfect LD and therefore pruned prior to analysis to reduce the multiple testing issue.

5.7.2.1 Genotypes

Table 5.10. Allele frequencies, base pair position and $R^2$ value for imputation for \textit{PER2} single nucleotide polymorphisms in OSA cases ($n=1,053$)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position bp</th>
<th>Major allele Base</th>
<th>Frequency</th>
<th>Minor allele Base</th>
<th>Frequency</th>
<th>hMAF</th>
<th>$R^2$</th>
<th>Genotyped/Imputed</th>
</tr>
</thead>
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<td>rs881933</td>
<td>238815780</td>
<td>G</td>
<td>67.07</td>
<td>C</td>
<td>32.93</td>
<td>26.9</td>
<td>0.91959</td>
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<tr>
<td>rs934945</td>
<td>238819792</td>
<td>C</td>
<td>81.15</td>
<td>T</td>
<td>18.85</td>
<td>15.9</td>
<td>0.97539</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs880140</td>
<td>238820471</td>
<td>G</td>
<td>94.61</td>
<td>A</td>
<td>5.39</td>
<td>8.90</td>
<td>0.97719</td>
<td>-</td>
</tr>
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<td>rs4663868</td>
<td>238825830</td>
<td>C</td>
<td>92.91</td>
<td>T</td>
<td>7.09</td>
<td>11.1</td>
<td>0.99876</td>
<td>-</td>
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<td>rs4429421#</td>
<td>238829172</td>
<td>C</td>
<td>99.62</td>
<td>T</td>
<td>0.38</td>
<td>0.80</td>
<td>0.22014</td>
<td>-</td>
</tr>
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<td>rs6431590</td>
<td>238829867</td>
<td>A</td>
<td>69.71</td>
<td>G</td>
<td>30.29</td>
<td>22.7</td>
<td>0.99694</td>
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<tr>
<td>rs2304670</td>
<td>238830375</td>
<td>C</td>
<td>92.69</td>
<td>T</td>
<td>7.31</td>
<td>11.9</td>
<td>0.99807</td>
<td>-</td>
</tr>
<tr>
<td>rs2304669</td>
<td>238830402</td>
<td>T</td>
<td>85.70</td>
<td>C</td>
<td>14.3</td>
<td>13.7</td>
<td>1.00011</td>
<td>-</td>
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<tr>
<td>rs10198215*</td>
<td>238830648</td>
<td>A</td>
<td>69.72</td>
<td>G</td>
<td>30.28</td>
<td>32.1</td>
<td>0.99603</td>
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<td>238834690</td>
<td>C</td>
<td>98.15</td>
<td>T</td>
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<td>1.80</td>
<td>0.86315</td>
<td>-</td>
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<td>rs7570188</td>
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<td>93.17</td>
<td>G</td>
<td>6.83</td>
<td>5.40</td>
<td>0.98593</td>
<td>-</td>
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<tr>
<td>rs10201361*</td>
<td>238838644</td>
<td>G</td>
<td>98.38</td>
<td>C</td>
<td>1.62</td>
<td>1.60</td>
<td>0.80358</td>
<td>-</td>
</tr>
<tr>
<td>rs4663299**</td>
<td>238839814</td>
<td>C</td>
<td>92.97</td>
<td>G</td>
<td>7.03</td>
<td>8.50</td>
<td>1.00038</td>
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<td>rs3739064</td>
<td>238841125</td>
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<td>78.58</td>
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<td>238841812</td>
<td>C</td>
<td>76.88</td>
<td>T</td>
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<td>21.7</td>
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<td>238843141</td>
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<td>69.84</td>
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<td>30.16</td>
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<td>T</td>
<td>92.96</td>
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SNP, Single nucleotide polymorphism; hMAF, Minor allele frequency reported using the International HapMap project: Data release 28, Phase II & III, August 10, on NCBI B36 assembly, dbSNP b126; bp, base pair position number on chromosome 2; *SNP pruned due to perfect linkage disequilibrium (LD), where $r^2=1$ and DPrime=1, N=7, Link for SNAP software: \url{http://www.broadinstitute.org/mpg/snap/ldsearchpw.php}; # MAF<0.1, SNP pruned.
Table 5.10a. Allele frequencies, base pair position and $R^2$ value for imputation of PER3 single nucleotide polymorphisms in OSA cases (n=1,053)

<table>
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<th>Frequency</th>
<th>Minor allele Base</th>
<th>Frequency</th>
<th>hMAF</th>
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<th>Genotyped/Imputed</th>
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SNP, Single nucleotide polymorphism; hMAF, Minor allele frequency reported using the International HapMap project: Data release 28, Phase II & III, August 10, on NCBI B36 assembly, dbSNP b126; bp, base pair position number on chromosome 1; *SNP pruned due to perfect linkage disequilibrium (LD), where $r^2=1$ and DPrime=1, N=8.
Table 5.10b continued. Allele frequencies, base pair position and $R^2$ value for imputation of PER3 single nucleotide polymorphisms in OSA cases (n=1,053)

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SNP, Single nucleotide polymorphism; hMAF, Minor allele frequency reported using the International HapMap project: Data release 28, Phase II & III, August 10, on NCBI B36 assembly, dbSNP b126; bp, base pair position number on chromosome 1; *SNP pruned due to perfect linkage disequilibrium (LD), where $r^2=1$ and DPrime=1, N=19.
5.7.2.2 Results of multivariate analyses for ESS continuous and all \textit{PER2} SNPs

The results presented in the following tables represent the complete details for all \textit{PER2} and \textit{PER3} SNPs analysed in this study.

\textbf{Supplementary Table 5.11.} Results of multivariate analyses for Epworth Sleepiness Score and all \textit{PER2} SNPs in the study *

\begin{tabular}{lcccc}
SNP & $\beta$ coefficient & Standard Error & Lower 95\% CI & Upper 95\% CI & SNP p value \\
\hline
rs4663302 & -0.331 & 0.242 & -0.804 & 0.143 & 0.171 \\
rs11894535 & 0.293 & 0.271 & -0.239 & 0.825 & 0.281 \\
rs2304673 & 0.317 & 0.325 & -0.320 & 0.953 & 0.329 \\
rs1972874 & 0.206 & 0.247 & -0.279 & 0.690 & 0.406 \\
rs11892306 & 0.205 & 0.248 & -0.282 & 0.692 & 0.410 \\
rs2304674 & 0.152 & 0.254 & -0.347 & 0.650 & 0.551 \\
rs7570188 & 0.265 & 0.460 & -0.637 & 1.167 & 0.565 \\
rs3739064 & 0.146 & 0.279 & -0.400 & 0.692 & 0.601 \\
rs6431590 & 0.120 & 0.246 & -0.361 & 0.602 & 0.624 \\
rs934945 & -0.138 & 0.284 & -0.694 & 0.418 & 0.627 \\
rs10462023 & 0.114 & 0.236 & -0.349 & 0.576 & 0.630 \\
rs2304669 & 0.133 & 0.326 & -0.506 & 0.771 & 0.683 \\
rs2304677 & 0.206 & 0.560 & -0.891 & 1.302 & 0.713 \\
rs2304672 & -0.138 & 0.448 & -1.015 & 0.740 & 0.758 \\
rs2304670 & -0.128 & 0.441 & -0.993 & 0.736 & 0.772 \\
rs4663868 & -0.120 & 0.447 & -0.996 & 0.755 & 0.788 \\
rs880140 & 0.130 & 0.509 & -0.868 & 1.128 & 0.798 \\
rs2304676 & 0.123 & 0.647 & -1.146 & 1.392 & 0.849 \\
rs881933 & -0.032 & 0.249 & -0.520 & 0.457 & 0.899 \\
rs13382977 & 0.094 & 0.910 & -1.689 & 1.878 & 0.917 \\
\end{tabular}

* Model adjusted for age, sex, body mass index, caffeine and alcohol use; SNP, Single nucleotide polymorphism;
### Results of multivariate analyses for ESS continuous and all PER3 SNPs

Supplementary Table 5.12. Results of multivariate analyses for Epworth Sleepiness Score and all PER3 SNPs (N = 30) in the study *

<table>
<thead>
<tr>
<th>SNP</th>
<th>β coefficient</th>
<th>Standard error</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>SNP p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs228689</td>
<td>-0.751</td>
<td>0.415</td>
<td>-1.564</td>
<td>0.063</td>
<td>0.071</td>
</tr>
<tr>
<td>rs228682</td>
<td>-0.354</td>
<td>0.229</td>
<td>-0.804</td>
<td>0.095</td>
<td>0.123</td>
</tr>
<tr>
<td>rs10864316</td>
<td>0.390</td>
<td>0.295</td>
<td>-0.188</td>
<td>0.969</td>
<td>0.186</td>
</tr>
<tr>
<td>rs875994</td>
<td>0.389</td>
<td>0.307</td>
<td>-0.216</td>
<td>0.989</td>
<td>0.209</td>
</tr>
<tr>
<td>rs12736878</td>
<td>1.510</td>
<td>1.208</td>
<td>-0.857</td>
<td>3.877</td>
<td>0.212</td>
</tr>
<tr>
<td>rs707463</td>
<td>0.244</td>
<td>0.236</td>
<td>-0.218</td>
<td>0.706</td>
<td>0.301</td>
</tr>
<tr>
<td>rs696306</td>
<td>0.236</td>
<td>0.240</td>
<td>-0.233</td>
<td>0.706</td>
<td>0.324</td>
</tr>
<tr>
<td>rs707465</td>
<td>0.220</td>
<td>0.236</td>
<td>-0.244</td>
<td>0.683</td>
<td>0.353</td>
</tr>
<tr>
<td>rs707467</td>
<td>0.239</td>
<td>0.290</td>
<td>-0.329</td>
<td>0.807</td>
<td>0.409</td>
</tr>
<tr>
<td>rs228729</td>
<td>-0.191</td>
<td>0.242</td>
<td>-0.664</td>
<td>0.283</td>
<td>0.430</td>
</tr>
<tr>
<td>rs228692</td>
<td>-0.375</td>
<td>0.501</td>
<td>-1.358</td>
<td>0.607</td>
<td>0.454</td>
</tr>
<tr>
<td>rs11579477</td>
<td>1.295</td>
<td>1.815</td>
<td>-2.262</td>
<td>4.853</td>
<td>0.476</td>
</tr>
<tr>
<td>rs4908482</td>
<td>0.165</td>
<td>0.233</td>
<td>-0.292</td>
<td>0.621</td>
<td>0.479</td>
</tr>
<tr>
<td>rs228665</td>
<td>-0.161</td>
<td>0.242</td>
<td>-0.635</td>
<td>0.312</td>
<td>0.504</td>
</tr>
<tr>
<td>rs2172563</td>
<td>-0.175</td>
<td>0.272</td>
<td>-0.709</td>
<td>0.358</td>
<td>0.520</td>
</tr>
<tr>
<td>rs228669</td>
<td>-0.271</td>
<td>0.423</td>
<td>-1.100</td>
<td>0.557</td>
<td>0.521</td>
</tr>
<tr>
<td>rs11121023</td>
<td>-0.171</td>
<td>0.272</td>
<td>-0.705</td>
<td>0.363</td>
<td>0.530</td>
</tr>
<tr>
<td>rs697693</td>
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<td>0.276</td>
<td>-0.375</td>
<td>0.706</td>
<td>0.548</td>
</tr>
<tr>
<td>rs12035969</td>
<td>-0.163</td>
<td>0.273</td>
<td>-0.698</td>
<td>0.372</td>
<td>0.550</td>
</tr>
<tr>
<td>rs12025388</td>
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<td>-0.697</td>
<td>0.372</td>
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</tr>
<tr>
<td>rs11121030</td>
<td>-0.301</td>
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<td>-1.385</td>
<td>0.783</td>
<td>0.586</td>
</tr>
<tr>
<td>rs228642</td>
<td>0.124</td>
<td>0.228</td>
<td>-0.323</td>
<td>0.571</td>
<td>0.586</td>
</tr>
<tr>
<td>rs7550657</td>
<td>0.115</td>
<td>0.229</td>
<td>-0.333</td>
<td>0.564</td>
<td>0.614</td>
</tr>
<tr>
<td>rs228641</td>
<td>-0.218</td>
<td>0.568</td>
<td>-1.331</td>
<td>0.895</td>
<td>0.702</td>
</tr>
<tr>
<td>rs228700</td>
<td>-0.200</td>
<td>0.528</td>
<td>-1.234</td>
<td>0.834</td>
<td>0.705</td>
</tr>
<tr>
<td>rs228664</td>
<td>-0.187</td>
<td>0.571</td>
<td>-1.306</td>
<td>0.932</td>
<td>0.744</td>
</tr>
<tr>
<td>rs1012477</td>
<td>0.094</td>
<td>0.315</td>
<td>-0.523</td>
<td>0.711</td>
<td>0.766</td>
</tr>
<tr>
<td>rs2640909</td>
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<td>0.263</td>
<td>-0.498</td>
<td>0.532</td>
<td>0.948</td>
</tr>
<tr>
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<td>0.649</td>
<td>-1.309</td>
<td>1.235</td>
<td>0.955</td>
</tr>
<tr>
<td>rs1891217</td>
<td>0.004</td>
<td>0.437</td>
<td>-0.852</td>
<td>0.860</td>
<td>0.992</td>
</tr>
</tbody>
</table>

* Model adjusted for age, sex, body mass index, caffeine and alcohol use; SNP, Single nucleotide polymorphism
5.7.2.4  Results of multivariate analyses for Somnificity and all PER2 SNPs

**Supplementary Table 5.13.** Results of multivariate analyses for Somnificity (risk of dozing) and all PER2 SNPs in the study #

<table>
<thead>
<tr>
<th>SNP</th>
<th>β coefficient</th>
<th>Standard Error</th>
<th>Odds Ratio</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>SNP p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10462023</td>
<td>0.203</td>
<td>0.098</td>
<td>1.226</td>
<td>1.011</td>
<td>1.487</td>
<td>0.039</td>
</tr>
<tr>
<td>rs7570188</td>
<td>-0.263</td>
<td>0.185</td>
<td>0.769</td>
<td>0.535</td>
<td>1.105</td>
<td>0.155</td>
</tr>
<tr>
<td>rs6431590</td>
<td>-0.142</td>
<td>0.101</td>
<td>0.868</td>
<td>0.713</td>
<td>1.057</td>
<td>0.159</td>
</tr>
<tr>
<td>rs881933</td>
<td>-0.125</td>
<td>0.102</td>
<td>0.883</td>
<td>0.723</td>
<td>1.078</td>
<td>0.222</td>
</tr>
<tr>
<td>rs934945</td>
<td>-0.129</td>
<td>0.116</td>
<td>0.879</td>
<td>0.700</td>
<td>1.103</td>
<td>0.265</td>
</tr>
<tr>
<td>rs4663302</td>
<td>-0.104</td>
<td>0.099</td>
<td>0.901</td>
<td>0.742</td>
<td>1.095</td>
<td>0.296</td>
</tr>
<tr>
<td>rs1972874</td>
<td>-0.103</td>
<td>0.101</td>
<td>0.902</td>
<td>0.740</td>
<td>1.100</td>
<td>0.309</td>
</tr>
<tr>
<td>rs11892306</td>
<td>-0.095</td>
<td>0.102</td>
<td>0.910</td>
<td>0.745</td>
<td>1.111</td>
<td>0.353</td>
</tr>
<tr>
<td>rs2304677</td>
<td>0.199</td>
<td>0.237</td>
<td>1.221</td>
<td>0.767</td>
<td>1.942</td>
<td>0.400</td>
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<td>rs11894535</td>
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<td>0.111</td>
<td>0.912</td>
<td>0.734</td>
<td>1.133</td>
<td>0.407</td>
</tr>
<tr>
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<td>0.364</td>
<td>1.549</td>
<td>0.438</td>
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<td>0.854</td>
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<td>1.277</td>
<td>0.442</td>
</tr>
<tr>
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<td>0.104</td>
<td>0.937</td>
<td>0.764</td>
<td>1.150</td>
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<tr>
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<td>0.183</td>
<td>0.910</td>
<td>0.635</td>
<td>1.303</td>
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<tr>
<td>rs2304672</td>
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<td>0.184</td>
<td>0.912</td>
<td>0.636</td>
<td>1.308</td>
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<tr>
<td>rs2304676</td>
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<td>0.905</td>
<td>0.543</td>
<td>1.508</td>
<td>0.701</td>
</tr>
<tr>
<td>rs2304670</td>
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<td>0.935</td>
<td>0.655</td>
<td>1.335</td>
<td>0.712</td>
</tr>
<tr>
<td>rs2304669</td>
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<tr>
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<td>0.134</td>
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<td>1.352</td>
<td>0.773</td>
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<td>0.115</td>
<td>0.980</td>
<td>0.783</td>
<td>1.228</td>
<td>0.863</td>
</tr>
</tbody>
</table>

# Model adjusted for age, sex, body mass index, caffeine and alcohol use; SNP, Single nucleotide polymorphism
5.7.2.5 Results of multivariate analyses for Somnificity and all *PER3* SNPs

**Supplementary Table 5.14.-** Results of multivariate analyses for Somnificity (risk of dozing) and all *PER3* SNPs (N = 30) in the study *

<table>
<thead>
<tr>
<th>SNP</th>
<th>ß coefficient</th>
<th>Standard Error</th>
<th>Odds Ratio</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>SNP p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs707463</td>
<td>0.139</td>
<td>0.098</td>
<td>1.149</td>
<td>0.949</td>
<td>1.392</td>
<td>0.155</td>
</tr>
<tr>
<td>rs228669</td>
<td>-0.239</td>
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<td>0.787</td>
<td>0.562</td>
<td>1.104</td>
<td>0.166</td>
</tr>
<tr>
<td>rs707467</td>
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<td>0.120</td>
<td>1.174</td>
<td>0.927</td>
<td>1.487</td>
<td>0.182</td>
</tr>
<tr>
<td>rs707465</td>
<td>0.124</td>
<td>0.098</td>
<td>1.132</td>
<td>0.935</td>
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<td>0.205</td>
</tr>
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<td>1.133</td>
<td>0.934</td>
<td>1.376</td>
<td>0.206</td>
</tr>
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<td>0.755</td>
<td>0.479</td>
<td>1.189</td>
<td>0.225</td>
</tr>
<tr>
<td>rs228641</td>
<td>-0.272</td>
<td>0.231</td>
<td>0.762</td>
<td>0.485</td>
<td>1.198</td>
<td>0.239</td>
</tr>
<tr>
<td>rs228729</td>
<td>-0.113</td>
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<td>0.893</td>
<td>0.735</td>
<td>1.085</td>
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</tr>
<tr>
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<td>0.094</td>
<td>0.901</td>
<td>0.749</td>
<td>1.085</td>
<td>0.271</td>
</tr>
<tr>
<td>rs10864316</td>
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<td>1.125</td>
<td>0.884</td>
<td>1.431</td>
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<td>rs12738878</td>
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<td>1.640</td>
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<td>4.667</td>
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<td>1.225</td>
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<td>1.239</td>
<td>0.453</td>
</tr>
<tr>
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<td>1.081</td>
<td>0.865</td>
<td>1.351</td>
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<td>rs4908482</td>
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<td>1.061</td>
<td>0.879</td>
<td>1.280</td>
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</tr>
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<td>1.377</td>
<td>0.581</td>
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<td>0.521</td>
<td>1.480</td>
<td>0.626</td>
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<td>0.915</td>
<td>0.585</td>
<td>1.429</td>
<td>0.695</td>
</tr>
<tr>
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<td>0.094</td>
<td>1.032</td>
<td>0.858</td>
<td>1.241</td>
<td>0.740</td>
</tr>
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<td>0.966</td>
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<td>1.258</td>
<td>0.285</td>
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</tr>
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<td>rs11121023</td>
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<td>0.777</td>
<td>1.204</td>
<td>0.766</td>
</tr>
<tr>
<td>rs12035969</td>
<td>-0.028</td>
<td>0.112</td>
<td>0.973</td>
<td>0.781</td>
<td>1.212</td>
<td>0.805</td>
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<tr>
<td>rs12025388</td>
<td>-0.027</td>
<td>0.112</td>
<td>0.973</td>
<td>0.781</td>
<td>1.212</td>
<td>0.807</td>
</tr>
<tr>
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<td>0.006</td>
<td>0.130</td>
<td>1.007</td>
<td>0.781</td>
<td>1.297</td>
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</table>

* Model adjusted for age, sex, body mass index, caffeine and alcohol use; SNP, Single nucleotide polymorphism
5.7.2.6 Multivariate results for association between \( \text{PER3} \) SNPs and ESS continuous, adjusting for AHI and an AHI*SNP interaction

**Supplementary Table 5.15.** Results of multivariate analyses for ESS adjusted for AHI and AHI*SNP interactions for all \( \text{PER3} \) SNPs (\( n = 30 \)) *

<table>
<thead>
<tr>
<th>SNP</th>
<th>( \beta ) coefficient</th>
<th>Standard Error</th>
<th>( P ) value</th>
<th>SNP</th>
<th>( \beta ) coefficient</th>
<th>Standard Error</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
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<td>SNPs</td>
<td>-1.85409</td>
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<td>0.00962</td>
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<tr>
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<td>-4.39880</td>
<td>0.07971</td>
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<tr>
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<td>SNPs</td>
<td>-1.48002</td>
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<td>SNPs</td>
<td>-1.50034</td>
<td>0.05366</td>
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<td>0.01058</td>
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*Model adjusted for age, sex, body mass index, caffeine and alcohol use, AHI and an AHI*SNP interaction; SNP, Single nucleotide polymorphism*
5.7.2.7 Multivariate results for association between \textit{PER3} SNPs and Somnificity, adjusting for AHI and an AHI*SNP interaction

**Supplementary Table 5.16.** Results of multivariate analyses for Somnificity (risk of dozing) adjusted for AHI and AHI*SNP interactions for all \textit{PER3} SNPs (n = 30)

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# Model adjusted for age, sex, body mass index, caffeine and alcohol use, AHI and an AHI*SNP interaction; SNP, Single nucleotide polymorphism
6 A COMPREHENSIVE EVALUATION OF A TWO-CHANNEL PORTABLE MONITOR TO ‘RULE-IN’ OBSTRUCTIVE SLEEP APNOEA

6.1 FOREWORD

The study described in Chapter 5 shows that some genetic variants thought to be related to circadian rhythm control may predict vulnerability to the sleep disruption of OSA. This relationship was modulated by an interaction between the genetic variant and the primary exposure variable AHI, a measure of severity of OSA. This vulnerability to sleep disruption differed according to the genotype of a specific PER3 variant (rs697693).

The clinical implications of this finding are that, in the future, genetic variants may serve as biomarkers for individual vulnerability to sleep loss. For individuals with OSA, there is wide heterogeneity in the degree of daytime sleepiness and cognitive dysfunction that correlates poorly with disease severity. Past research has shown that untreated OSA is associated with an increased risk of driving\textsuperscript{16} and occupational accidents.\textsuperscript{429} The study in Chapter 3 confirmed this finding in our sleep clinic patients and also showed that there is a strong association between excessive daytime sleepiness and increased report of near-miss crashes. Furthermore, a systematic review and meta-analysis has concluded that treatment of OSA with CPAP reduces crash risk among drivers with OSA.\textsuperscript{253} When there is a high clinical suspicion of drowsy driving at first presentation, there is an imperative for the sleep physician to expedite diagnosis and treatment of OSA.
Obstructive Sleep Apnoea

The gold standard investigation of laboratory-based PSG is under-resourced in Australia relative to the high prevalence of undiagnosed OSA in the community.\textsuperscript{9,137} Where there is a high pre-test probability of OSA, home sleep tests conducted with a portable sleep device have the potential to expedite diagnosis and treatment, and so ameliorate driving risk. Limited-channel sleep studies are widely used in many countries such as the UK,\textsuperscript{430} but professional societies and government have been reluctant to endorse their routine use due to limited evidence of validity compared with the gold standard. To date, simple two-channel sleep monitors (Type 4) have not been validated to consistently rule-in OSA accurately. The final goal of this thesis was to validate a simple portable monitor for home sleep studies. A simple cost-effective evaluation of OSA with a home sleep study has the potential to allow triage of patients for urgent assessment and treatment. The research described in this chapter has been published in the \textit{Journal of Clinical Sleep Medicine}.\textsuperscript{431}

6.2 Abstract

\textbf{Study Objectives:} We hypothesised that a dual-channel portable monitor (PM) could accurately identify patients who have a high pre-test probability of obstructive sleep apnoea (OSA), and we evaluated factors that may contribute to variability between PM and polysomnography (PSG) results.

\textbf{Methods:} Consecutive clinic patients (n = 104) with possible OSA completed a home PM study, a PM study simultaneous with laboratory PSG and a second home PM study. Uniform data analysis methods were applied to both PM and PSG data. Primary outcomes of interest were the positive likelihood ratio (LR+) and
sensitivity of the PM to ‘rule-in’ OSA, defined as an apnoea-hypopnoea index (AHI) \(\geq 5\) events/h on PSG. Effects of different test environment and study nights, order of study and analysis methods (manual compared to automated) on PM diagnostic accuracy were assessed.

**Results:** The PM has adequate LR+ (4.8), sensitivity (80%) and specificity (83%) for detecting OSA in the unattended home setting when benchmarked against laboratory PSG, with better LR+ (>5) and specificity (100%), and unchanged sensitivity (80%) in the simultaneous laboratory comparison. There were no significant night-night (all \(p > 0.10\)) or study order effects (home or laboratory first, \(p = 0.08\)) on AHI measures. Manual PM data review improved case finding accuracy, although this was not statistically significant (all \(p > 0.07\)). Misclassification was more frequent where OSA was mild.

**Conclusions:** Overall performance of the PM is consistent with current recommended criteria for an ‘acceptable’ device to confidently ‘rule-in’ OSA (AHI \(\geq 5\) events/h) in a high pre-test probability clinic population. Our data support the utility of simple two-channel diagnostic devices to confirm the diagnosis of OSA in the home environment.
6.3 INTRODUCTION

Obstructive sleep apnoea (OSA) is a prevalent condition that is commonly associated with obesity, hypertension, habitual snoring and hypersomnolence. A recent update of the Wisconsin cohort study reported a disturbing increase in estimated prevalence of OSA over the last two decades, with population ageing and increasing obesity likely driving influences. Growing demand for access to diagnosis and treatment has led to longer waiting lists as the need for these services exceeds capacity. Population-based studies estimate that 90% of cases in the communities of advanced economies remain undiagnosed and untreated.

An important limiting factor has been a lack of access to and perceived expense of laboratory polysomnography (PSG), the current ‘gold standard’ for OSA diagnosis. There is an urgent need to research novel diagnostic methodologies that are less expensive and more widely applicable than PSG. Because of these difficulties many physicians have resorted to the use of ambulatory diagnostic devices, despite limited evidence of their accuracy.

Several recent reviews have assessed portable monitors (PMs) in OSA diagnosis. Identified shortcomings of the assessment of these devices include frequent failure to evaluate their effectiveness in their intended home setting, low patient numbers, inadequate randomisation of the order in which at-home and in-laboratory studies were made, and reliance on automated scoring of the data generated. In response to these deficiencies, Flemons et al. developed a system for grading the evidence from such studies and made recommendations.
regarding the use of appropriate research methods and reporting for the validation of PMs to minimise bias.\textsuperscript{43,435} All subsequent reviews have applied this grading methodology in order to build an evidence basis regarding the place of PM in the diagnosis of OSA.

The limitations outlined above appear to relate particularly to simple one or two channel (‘Type 4’) devices. While ‘Type 3’ PMs (which include 4 or more cardio-respiratory channels) have been approved for objective testing in several situations, the American Academy of Sleep Medicine (AASM) holds that there is insufficient evidence to support use of ‘Type 4’ PMs in unattended settings.\textsuperscript{116,127} Given the simplicity and relatively low cost of such devices, interest remains in definitively determining their place in diagnostic testing, for both clinical and research purposes.

Barriers to acceptance of simple PMs are their lack of an accurate measure of time asleep and inability to detect arousals, and therefore arousal-related respiratory events. It has been argued that clinicians could accept an index generated by a PM that may not agree completely with PSG if it accurately categorised presence or absence of OSA.\textsuperscript{135} Based upon this premise, the sensitivity, specificity and positive likelihood ratio (LR+) appear to be the best statistical measures for identifying a clear cut-off for a PM-generated AHI that defines presence or absence of the disorder.\textsuperscript{135} Indeed, Collop \textit{et al.} devised specific criteria to apply to the PM result to ensure a sufficiently high post-test probability (> 95%) to confidently rule in OSA.\textsuperscript{45} Their approach focussed upon assessing study quality and statistical methodology that ensured PM diagnosis accurately
categorised OSA, allowing for the limitations inherent in their inability to detect and stage sleep.

The recent recommendations of Collop et al. regarding the standards that should be applied to evaluation of PMs informed our approach to this study. The primary aim of the present study was to assess the accuracy of a dual-channel PM (ApneaLink®) as a triaging tool for suspected OSA in a population referred to a specialist sleep clinic. We hypothesised that by addressing limitations in previous studies we could demonstrate that this simple Type 4 device could accurately ‘rule-in’ OSA in high pre-test probability patients. The methodological weaknesses of previous studies were addressed by ensuring adequate sample size, testing in both home and laboratory environments, and assessment of night to night variability and order effects. We also compared PM analysis using the computer-aided visual data review method with automated analysis for this PM, by uploading PM recordings into our PSG analysis platform. We postulated further that some of the misclassification reported in past studies may be due to analysis discrepancies.
6.4 METHODS

6.4.1 Participants

Eligible study participants were patients referred to a sleep disorders clinic (West Australian Sleep Disorders Research Institute [WASDRI], Sir Charles Gairdner Hospital) for investigation of suspected OSA. Inclusion criteria were: age 18-75 years, referral for investigation of possible OSA, scheduled diagnostic PSG, and ability to adhere to all study components. Exclusion criteria included unstable coronary syndromes, severe chronic airflow limitation (FEV1 < 50% predicted), uncontrolled congestive cardiac failure, morbid obesity (BMI > 40), neuromuscular disease, cognitive impairment/disability such that the PM study was difficult to administer, previous diagnosis of OSA and use of CPAP or oxygen therapy. The study was approved by the local Human Research Ethics Committee (No. 2007-032).

6.4.2 Study design

A prospective repeat study protocol (Figure 6.1) was used in which subjects completed a home PM study two weeks prior to PSG (P1), a PM study simultaneous with laboratory PSG (P2), and a home PM study after PSG (P3). Subjects were randomly assigned to complete all (P1, P2, P3) assessments (Group 1) or only P2 and P3 (Group 2).
The afternoon prior to P1, a sleep technologist issued the PM and instructed the participant in its correct fitting and use (education 10 minutes). The subject took the equipment home, wore it during a ‘usual’ night’s sleep and returned it by post. During simultaneous PM and PSG (P2), the nasal pressure signal was delivered to both sleep systems using a Y-piece in the nasal catheter, a methodology validated in previous studies, and the subject wore separate oximetry finger probes for the PM and PSG. At the conclusion of the PSG all subjects were given a PM and instructed about its correct use. The subject took the device home, repeated the PM study within 1-14 days of the PSG (P3) and returned it by post.

The PM study was judged acceptable if it was ≥ 4 hours duration and both flow and saturation data were present for ≥ 90% of the recording time. When a PM study was not acceptable on these grounds participants were invited to repeat the study. Of the total number of PM studies conducted (n = 356), there were 70 (19.7%) failed studies (with some subjects having more than one failed study, so that the total number of subjects with failed studies was 35). Of the 70 failed studies, 37 (10.4%) were patient-related failures (insufficient duration or compliance), 20 (5.6%) were due to administrative error (booking error), and 13 (3.7%) were technical (signal loss) failures. Of the 139 patients who gave informed written consent to participate, 35 subjects had ‘failed’ PM studies, which left 104 evaluable patients (Figure 6.1).
Figure 6.1. Recruitment flow diagram.

*Detail of ineligible subjects given in text. # Detail of incomplete data given in text.
6.4.3 Measurements

6.4.3.1 Portable monitor study (PM study)

The PM studies were undertaken using the ApneaLink Ox™ device (Firmware version 04.08, software version 8.00) which comprises a nasal flow signal (using a nasal cannula/pressure transducer system, recording the inverse square root of pressure as an index of flow [sample rate 100Hz]), and pulse oximetry (Nonin XPod 3012 with a Nonin 7000A finger probe [sample rate 1 Hz]; Nonin, Hudiksvall, Sweden). Details of linearization of the nasal pressure signal and processing of artefact in the pulse oximetry signal have been outlined in past validation studies.\(^{436,437,440,441}\)

Initial PM data analysis was automated (by ApneaLink™ software) with rules defined to match laboratory PSG settings.\(^{85}\) Manual data review used the PSG data analysis platform after importing ApneaLink™ signals (EDF format with removal of automated results). PM studies were de-identified and scored by two accredited sleep scientists who are members of the Board of Registered Polysomnographic Technologists (BRPT), who were blind to the PSG results. To assess scoring concordance between the scientists, a random sample of 10 studies was analysed by both scorers and the intraclass correlation coefficient for the apnoea hypopnoea index (AHI) was calculated.

An apnoea was defined as a decrease in airflow by 80% of baseline (duration 10-80s). A hypopnoea was defined as a decrease in airflow ≥ 30% of baseline plus a 3% desaturation or a reduction of airflow ≥ 50% of duration 10-100 s. The index
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definition for AHI derived from PM (AHI PM) was apnoeas plus hypopnoeas per recording hour.

6.4.3.2 Polysomnography (PSG)

Overnight laboratory-based PSG was performed using the Compumedics E-Series (PSG Online 2, Compumedics Ltd, Abbotsford, Australia). Sleep was documented by standard electroencephalographic (EEG), electro-oculographic (EOG) and electromyographic (EMG) criteria. Other measurements included electrocardiogram (ECG), nasal pressure, oronasal airflow (thermocouple), thoracic and abdominal inductance plethysmography, oximetry (Nonin XPod 3012 with a Nonin 7000A finger probe [sample rate 1 Hz]; Nonin, Hudiksvall, Sweden) and bi-lateral leg movements (piezo-electric sensors).

PSG studies were manually scored by sleep scientists according to the recommendations published by the AASM using Profusion 2 software (Compumedics Ltd, Abbotsford, Australia). Obstructive apnoeas were defined as the absence (decrease by 80% from baseline) of airflow for ≥ 10 seconds. Obstructive hypopnoeas were defined as a ≥ 50% decrease in airflow, or a clear but lesser decrease in airflow associated with either a 3% desaturation or an EEG arousal in the context of ongoing respiratory effort. In the case of PSG, AHI was defined as the number of apnoeas plus hypopnoeas per sleep hour (AHI PSG). OSA was defined as AHI ≥ 5 events/h with severity of OSA defined as: Nil = AHI < 5 events/h; mild = 5 ≤ AHI < 15 events/h; moderate = 15 ≤ AHI < 30 events/h; severe = AHI ≥ 30 events/h.
6.4.4 Data analysis

Sample size calculation was based upon preliminary data showing a standard deviation of the mean difference in AHI between a similar PM device (Micromesam™, single channel) and PSG methods of 11.5 events/h for 25 paired data sets. Assuming $\alpha = 0.05$ and $> 90\%$ power, we estimated 89 patients were required to detect an AHI difference of 4 events/h between methods (a level of discrimination that accounts for potential variability in the AHI attributable to home versus laboratory-based study differences). Allowing for data wastage of 17% (based on the previous study) we planned to complete 104 evaluable cases.

Baseline demographic and sleep data for the cohort were described as mean±SD, or median (interquartile range [IQR]) for skewed data.

The primary outcome of interest was the diagnostic accuracy of the PM to ‘rule-in’ OSA at an AHI $\geq 5$ events/h. Account was taken of factors known to have contributed to variability in past studies as follows: a) night to night consistency of PM results, b) study order effect, c) underestimation of PM results, and d) manual data review versus auto-analysis.

Validation analysis included calculation of sensitivity, specificity and positive and negative likelihood ratios (LR+, LR-), using the PSG as the reference standard. We applied the statistical guidelines recommended by Collop et al. whereby an acceptable PM is judged according to whether it can produce a LR+$\geq 5$ and a sensitivity of at least 0.825 at an in-laboratory AHI of $\geq 5$ events/h, assuming a pre-test probability of 80%. We calculated the expected LR+ to achieve a post-test
probability of > 95% in our clinic population using our known prevalence rates for mild, moderate and severe OSA (expected LR+: 1.8, 6.5 and >10).

The night-to-night consistency of PM results (Group 1, n = 52) was evaluated by four methods: mean night to night differences between grouped data, mean night to night differences between paired data, correlation between repeated results and Bland-Altman plots of paired measurements. Order of study effect was investigated by randomisation of subjects to two groups (PM first [n = 52] or PSG first [n = 52]) and calculation of group mean differences. The misclassification percentages (at AHI ≥ 5 events/h) between the groups were compared using Chi-square tests.

The effects of different study night, equipment and environment on AHI measured at home and in the laboratory were assessed using bivariate correlation, identity plots and Bland-Altman plots. Mean differences between AHI PSG and AHI PM for the cohort (P2 and P3, n = 104) were calculated.

Accuracy of manual analysis compared with auto-analysis was investigated by calculation of sensitivity, specificity and percentage of missed cases. The misclassification percentage for auto-analysis was compared with manual review by Chi-square analysis.

Data were analysed using SPSS statistics (GradPack 17.0 Release 17.0.2, March 11, 2009), and R software (Version 2.14.1, 2011-12-22). Statistical significance was defined at the 5% level.
6.5 RESULTS

Over a one year period, 223 subjects were approached to participate in the study. Of these subjects, 139 consented to participate and were randomised to receive either a PM home study first (Group 1) or a simultaneous laboratory PM and PSG study first (Group 2) (Figure 6.1).

6.5.1 Subject characteristics

Subjects were predominantly male (64%), middle-aged (50.7±13.5 yr), obese (BMI: 31.3±6.3 kg/m²) and commonly reported daytime sleepiness (ESS: 9.3 ± 5.6, Table 6.1). Subjects had moderately severe OSA (AHI: 28.5, 13.3-37.5 events/h) and evaluable subjects (n = 104) had a wide range of disease severity (AHI range: 1 to 129 events/h). The median minimum oxygen saturation was 88% (81-92) and time spent at an arterial oxygen saturation ≤ 90% was 0.6 minutes (0.0-10.7, Table 6.1). There were no significant differences between the demographic and sleep characteristics of subjects who were evaluable (n = 104) and those who weren’t (n = 35, Table 6.1). Scoring reliability between the two BRPT-accredited scorers was high, with intraclass correlation coefficient (ICC) values for P2 and P3 of 0.97 (95% CI 0.88, 0.99) and 0.98 (95% CI 0.70, 0.99). The prevalence of OSA in this study cohort is comparable to the prevalence in our tertiary referred clinic population (Table 6.2).
Table 6.1. Characteristics of the study cohort

<table>
<thead>
<tr>
<th>Subjects (n = 104)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, Male, n (%)</td>
<td>64 (62)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.3 ± 6.3</td>
</tr>
<tr>
<td>Age, yr</td>
<td>50.7 ± 13.5</td>
</tr>
<tr>
<td>ESS, score</td>
<td>9.3 ± 5.6</td>
</tr>
<tr>
<td>AHI, events/h</td>
<td>28.5 (13.3, 37.5)</td>
</tr>
<tr>
<td>ARI, events/h</td>
<td>34.2 (21.4, 48.0)</td>
</tr>
<tr>
<td>Minimum SaO₂, %</td>
<td>88 (81, 92)</td>
</tr>
<tr>
<td>Time spent &lt; 90% SaO₂, min</td>
<td>0.6 (0, 10.7)</td>
</tr>
</tbody>
</table>

PSG sleep parameters:

- Total recording time, min 472.9 ± 54.5
- Total sleep time, min 349.0 ± 78.8
- Sleep efficiency, % 74.2 ± 14.3
- Total time awake, min 123.9 ± 74.1

Data are presented as mean±SD or median (interquartile range); BMI, body mass index; ESS, Epworth Sleepiness Score; AHI, apnoea hypopnoea index; ARI, arousal index; SaO₂, arterial oxygen saturation; PSG, polysomnography.
Table 6.2. Prevalence of obstructive sleep apnoea in portable study cohort compared with Western Australian Sleep Health Study cohort

<table>
<thead>
<tr>
<th>Level of OSA</th>
<th>Study cohort n = 104</th>
<th>WASHS\textsuperscript{a} N = 2663</th>
<th>p value\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>6 (5.8)</td>
<td>179 (6.7)</td>
<td>0.72</td>
</tr>
<tr>
<td>Any OSA</td>
<td>98 (94.2)</td>
<td>2484 (93.3)</td>
<td>0.72</td>
</tr>
<tr>
<td>Moderate–severe</td>
<td>75 (72.1)</td>
<td>1904 (71.5)</td>
<td>0.88</td>
</tr>
<tr>
<td>Severe</td>
<td>51 (49.0)</td>
<td>1166 (43.8)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

OSA, obstructive sleep apnoea; Any OSA, AHI≥5 events/h; Moderate–severe OSA, AHI≥15 events/h; Severe OSA, AHI≥30 events/h; WASHS\textsuperscript{a}, Western Australian Sleep Health Study;\textsuperscript{33} P value\textsuperscript{b}, Pearson Chi-square test.

6.5.2 PM performance

There were no significant night to night variability or order effects for PM data (see ‘Potential sources of PM performance variability’, below); hence all data for P2 and P3 (ie. from Groups 1 and 2) were combined to optimise statistical power (n = 104) in the following correlational, agreement and diagnostic accuracy analyses.

6.5.2.1 Correlation between PM studies and PSG

Data from all evaluable subjects (n = 104) showed that there were generally good correlations between AHI PM from night to night and with laboratory AHI PSG (Figure 6.2, Panels A, B and C). The closest correlations were seen between tests on different equipment (PM and laboratory) done on the same night in the same laboratory environment (r = 0.9, Figure 6.2, Panel A), and between tests on the same equipment but different nights and different environments (r = 0.9, Figure 6.2, Panel B).
6.5.2.2 Agreement between PM studies and PSG

The AHI PM (home and laboratory) underestimated AHI PSG, and the difference between the two methods increased with the mean AHI PSG/AHI PM (Figure 6.2, Panels A and C). In panel A almost all data points fell below the line of identity in the identity plot and above the line of no difference in the Bland-Altman plot. The AHI PM on all three study nights was significantly lower (p < 0.001) than AHI PSG. Mean differences ranged from 13.5 events/h (95% CI 11.1, 15.9) on the simultaneous night (P2) to 17.2 events/h (95% CI 12.0, 22.4) on the pre-PSG night (P1), and 14.8 events/h (95% CI 11.8, 17.8) on the post-PSG night (P3). By contrast, agreement was best using the same equipment, on a different night but in the same environment (Figure 6.2B, Bland-Altman plot and Figure 6.3).
Figure 6.2. Identity and Bland-Altman plots comparing AHI for in-lab PSG with AHI for PM studies, \(n = 104\), composed of all data from P2 and P3 nights (ie. Group 1 and Group 2 combined).

(A) Simultaneous recordings (same night and environment, different equipment)
(B) Compares PM studies done in-lab and at home (different night and environment, same equipment) 
(C) Compares in-lab PSG with home PM study (different night, environment and equipment)
Figure 6.3. Comparisons of AHI for PM before PSG (P1), PM simultaneous with PSG (P2) and PM after PSG (P3) for Group 1 subjects (N=52).

(A) P1 versus P2 (r=0.80, p=0.14) (B) P3 versus P2 (r=0.87, p=0.63) (C) P1 versus P3, (r=0.84, p=0.26)
6.5.2.3 Diagnostic accuracy of the PM

Table 6.3 presents data for the diagnostic accuracy of the PM to categorise mild, moderate and severe OSA for simultaneous data collection (P2) and in the unattended home setting (P3). The PM had good diagnostic accuracy to rule in OSA (AHI ≥ 5 events/h) with a sensitivity of 80% and LR+ of 4.8 in the home setting (Table 6.3). Positive LR remained high (infinity, due to the denominator of 1-specificity being zero) to ‘rule-in’ both moderate and severe OSA, but there was a progressive loss of sensitivity (66%, 43% respectively).

Table 6.3. Diagnostic accuracy of combined group 1 and 2 data for study nights 2 and 3 using the portable monitor relative to clinical standard polysomnography for mild, moderate and severe obstructive sleep apnoea

<table>
<thead>
<tr>
<th>Study Night</th>
<th>Pre-test Prob^</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>+LR</th>
<th>-LR</th>
<th>Post-test Prob*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any OSA P2</td>
<td>0.93</td>
<td>0.80 (0.71, 0.88)</td>
<td>1.00 #</td>
<td>∞</td>
<td>0.20</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>AHI ≥ 5 events/h P3</td>
<td>0.80 (0.72, 0.89)</td>
<td>0.83 (0.76, 0.91)</td>
<td>4.8</td>
<td>0.23</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Moderate OSA P2</td>
<td>0.71</td>
<td>0.74 (0.63, 0.84)</td>
<td>1.00 #</td>
<td>∞</td>
<td>0.26</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>AHI ≥ 15 events/h P3</td>
<td>0.66 (0.55, 0.77)</td>
<td>1.00 #</td>
<td>∞</td>
<td>0.34</td>
<td>&gt;0.99</td>
<td></td>
</tr>
<tr>
<td>Severe OSA P2</td>
<td>0.44</td>
<td>0.50 (0.36, 0.64)</td>
<td>1.00 #</td>
<td>∞</td>
<td>0.50</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>AHI ≥ 30 events/h P3</td>
<td>0.43 (0.28, 0.57)</td>
<td>0.98 (0.94, 1.02)</td>
<td>21.7</td>
<td>0.59</td>
<td>0.94</td>
<td></td>
</tr>
</tbody>
</table>

OSA, obstructive sleep apnoea; ^Pre-test Prob, Pre-test probability; *Post-test Prob, post-test probability; AHI, apnoea hypopnoea index; P2, portable simultaneous with PSG; P3, portable post-PSG; +LR, positive likelihood ratio; -LR, negative likelihood ratio; #, 95% CI not relevant since specificity is 100%.
6.5.3 **Potential sources of PM performance variability**

6.5.3.1 **Night to night variability**

Group 1 subjects (n = 52) had three PM studies (home P1, lab P2, home P3). Identity plots showed good correlation between the paired comparisons (r = 0.80 to 0.87) for Group 1 subjects (Figure 6.3). The mean night to night differences between home PM study results were small (AHI P1-P3: -1.6, 95% CI -4.4, 1.22, p = 0.26). Comparison between the home PM studies (P1 and P3) and laboratory PM study (P2) showed small mean differences (AHI P1-P2: -2.84, 95% CI -6.7, 1.0, p = 0.14, and AHI P3-P2: 0.72, 95% CI -2.2, 3.7, p = 0.63) of a similar magnitude. Similarly, Bland-Altman plots showed strong agreement between repeated PM results with mean differences close to zero and ranging from -2.8 to 1.5 events/h (Figure 6.3).

6.5.3.2 **Order effect**

The study design enabled exploration of the potential effect of order of measurement method. Group 1 subjects had a home PM study first (P1), while Group 2 subjects had laboratory PSG first, followed by home PM study (P3). The group mean difference between AHI PSG and home AHI PM was small (mean difference 3.2, 95% CI -3.2, 9.5) and there was no significant difference (p = 0.33) between the mean differences for the groups based upon order of study. Analysis of accuracy of classification (at AHI ≥ 5 events/h) for both groups found 11% (n = 5) misclassification for PM first subjects compared with 24% (n = 12) misclassification for PSG first subjects, but the difference in these proportions was
not significantly different ($p = 0.08$). Misclassification was more frequent where OSA was mild.

6.5.3.3 Comparison of automated analysis with manual data review

Seventy five (72%) subjects had moderate to severe OSA on PSG (Table 6.2). Manual review correctly classified 55 cases (73%) while auto-analysis correctly classified 46 cases (61%) (Table 6.4). Thus 9 cases (12%) of moderate-severe OSA were missed by the auto-analysis of the PM data. Table 6.4 shows that at every AHI level, manual review of data reduced the percentage of missed cases by 4% to 18%, irrespective of whether data were collected simultaneously in the laboratory or in the unattended home setting. However, these reductions were not statistically significant (all $p > 0.07$, Table 6.4).
**Table 6.4.** Accuracy of manual analysis compared with auto-analysis for mild, moderate and severe obstructive sleep apnoea

<table>
<thead>
<tr>
<th>OSA category</th>
<th>Study night</th>
<th>Analysis Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>n (%)</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any OSA</td>
<td>P2</td>
<td>Automated</td>
<td>0.76</td>
<td>1.00</td>
<td>24 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHI ≥ 5 events/h</td>
<td>Manual</td>
<td></td>
<td>0.80</td>
<td>1.00</td>
<td>20 (20)</td>
<td>0.49</td>
<td>4</td>
</tr>
<tr>
<td>(n = 98)</td>
<td>P3</td>
<td>Automated</td>
<td>0.76</td>
<td>0.83</td>
<td>24 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Manual</td>
<td>0.80</td>
<td>0.83</td>
<td>19 (20)</td>
<td>0.39</td>
<td>5</td>
</tr>
<tr>
<td>Moderate OSA</td>
<td>P2</td>
<td>Automated</td>
<td>0.61</td>
<td>0.96</td>
<td>29 (39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHI ≥ 15 events/h</td>
<td>Manual</td>
<td></td>
<td>0.74</td>
<td>1.00</td>
<td>20 (26)</td>
<td>0.12</td>
<td>12</td>
</tr>
<tr>
<td>(n = 75)</td>
<td>P3</td>
<td>Automated</td>
<td>0.57</td>
<td>1.00</td>
<td>32 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Manual</td>
<td>0.66</td>
<td>1.00</td>
<td>26 (34)</td>
<td>0.31</td>
<td>8</td>
</tr>
<tr>
<td>Severe OSA</td>
<td>P2</td>
<td>Automated</td>
<td>0.33</td>
<td>0.98</td>
<td>34 (67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHI ≥ 30 events/h</td>
<td>Manual</td>
<td></td>
<td>0.50</td>
<td>1.00</td>
<td>25 (50)</td>
<td>0.07</td>
<td>18</td>
</tr>
<tr>
<td>(n = 51)</td>
<td>P3</td>
<td>Automated</td>
<td>0.28</td>
<td>1.00</td>
<td>37 (72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Manual</td>
<td>0.43</td>
<td>0.98</td>
<td>29 (57)</td>
<td>0.10</td>
<td>16</td>
</tr>
</tbody>
</table>

OSA, obstructive sleep apnoea; AHI, apnoea hypopnoea index; P2, portable simultaneous with PSG; P3, portable post-PSG; Automated, automated analysis from ApneaLink™ software; Manual, manual review of raw data on PSG software; P value<sup>a</sup>, Pearson Chi-square test.
Table 6.5. Results from ApneaLink™ validation studies conducted in the recommended setting: portable monitor study simultaneous with laboratory polysomnography (L/L) and portable monitor study at home compared with laboratory polysomnography (H/L).

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Sample size (n)</th>
<th>OSA Prev (%)</th>
<th>AHI threshold (events/h)</th>
<th>Diagnostic accuracy Same night (L/L)</th>
<th>Different night (H/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sens</td>
<td>Spec</td>
</tr>
<tr>
<td>Present study</td>
<td>104</td>
<td>93</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.80</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>104</td>
<td>72</td>
<td>15</td>
<td>0.74</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>30</td>
<td></td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>Oktay 2011</td>
<td>53</td>
<td>76</td>
<td>5</td>
<td>0.90</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>36</td>
<td>15</td>
<td>0.79</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>30</td>
<td></td>
<td>0.67</td>
<td>0.96</td>
</tr>
<tr>
<td>Crowley 2013</td>
<td>48</td>
<td>41</td>
<td>5</td>
<td>0.89</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>16</td>
<td>15</td>
<td>1.00</td>
<td>0.92</td>
</tr>
<tr>
<td>Ragette 2010</td>
<td>102</td>
<td>80</td>
<td>5</td>
<td>0.94</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>131</td>
<td>49</td>
<td>15</td>
<td>0.92</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Study type: L/L, simultaneous laboratory PSG and PM study; H/L, laboratory PSG compared with home PM study; Device type, 2 channel, NP and oximetry; Manual review of raw data; OSA Prev: OSA prevalence at AHI thresholds of 5, 15 and 30; Sens: sensitivity; Spec: specificity; LR+: positive likelihood ratio.
6.6 DISCUSSION

This study confirms the accuracy of a two channel (Type 4) PM for the diagnosis of OSA in a sleep clinic population with a high pre-test probability of the disorder. We found the PM has an adequate LR+ (4.8) and sensitivity (80%) for OSA (AHI $\geq 5$ events/h) in the unattended home setting compared with laboratory PSG, and a better LR+ (infinity) and unchanged sensitivity (80%) in the simultaneous laboratory comparison. Hence, the overall performance of the device is consistent with the current recommended criteria for an ‘acceptable’ PM to confidently ‘rule-in’ OSA (AHI $\geq 5$ events/h) in a high pre-test probability clinic population.45

Unlike many preceding studies of PMs, this study was conducted according to the recommendations of expert working groups with both concurrent testing with laboratory PSG and testing at home, the intended setting for its use.43,116,128 We found no significant differences in night to night AHI measures, nor was there a study order effect (home or laboratory first) when comparing mean differences between groups. Misclassification did not differ significantly between groups (PM first 11% versus PSG first 24%, $p = 0.08$), but was more frequent where OSA was mild. Manual PM data review improved case finding accuracy, but the difference in accuracy did not reach statistical significance.

Previous studies have one or more of the following limitations: (a) failure to study the PM in its site of intended use, the home;43,439,444,445 (b) low sample size;436-441,444,445 (c) failure to collect oximetry data;436-440,444,446 (d) no randomisation of order of comparison;438,439,444,445 and (d) no manual review of the
raw PM data. Our validation addresses these limitations. It examines the PM in both the home and laboratory settings, is adequately powered, includes both flow and oximetry data, avoids order bias and assesses the impact of manual scoring of PM data on performance.

Some previous studies have validated this PM in the laboratory (i.e., concurrent with PSG) and reported good sensitivity (80-100%) to ‘rule-in’ OSA (AHI ≥ 5 events/h) but variable specificity (50-100%), with LR+ values ranging from 1.9 to infinity. Our study showed high diagnostic accuracy to ‘rule-in’ OSA with specificity 100% and LR+ infinity on the simultaneous night assessment. Other studies that have evaluated the device simultaneously with PSG in the laboratory and at home have shown less agreement on the home study night (Table 6.5). Simultaneous night data showed high sensitivity in all studies (89 to 94%) but specificity was variable and LR+ values ranged from 1.9 to 3.9. The comparisons of PSG with the home study night showed moderate sensitivity in one study (68%) and good sensitivity in two studies (81% and 92% respectively), comparable with the 80% observed in our study. Specificity was moderate in these previous studies (all 3 = 77%) compared with 83% in this study. Hence this study, which has carefully addressed the limitations of previously described studies, demonstrates that ApneaLink™ either meets (simultaneous laboratory comparison) or is close to meeting (home versus laboratory PSG comparison) the current recommended criteria for an ‘acceptable’ device to ‘rule-in’ OSA in the clinical setting. Note that single-channel oximetry using a 3% desaturation gave equivalent results to ‘rule-in’ moderate to severe OSA.
However, to ‘rule-in’ mild OSA (AHI ≥ 5 events/h) the addition of the nasal pressure signal resulted in better specificity and more cases were identified. Previous work published by our group has shown that oximetry alone is less helpful for lean patients. In patients with a low BMI, nasal pressure is likely to be a more discriminatory signal as this group will have less arterial oxygen desaturation for a given degree of upper airway obstruction.

Several authors have used identity and Bland-Altman plots to illustrate the inherent bias between AHI PM and AHI PSG on one hand, and good agreement between repeated PM studies on the other. Our data confirm that, even when studied with the same night and environment (Figure 6.2, Panel A), the PM consistently underestimates AHI PSG. The effect of a different study night is to increase the spread of data (Figure 6.2, Panel C; r = 0.84). By contrast, where the same equipment is used (Figure 6.2, Panel B), agreement is good despite the introduction of the potentially confounding variables of different night and environment. Thus the PM underestimated AHI PSG by 13.5 to 17.2 events/h, likely because of reliance on a different denominator (monitoring time for the PM versus sleep time for laboratory PSG) to calculate the AHI and/or the inability to score EEG arousal-related events for the PM study. This degree of underestimation is consistent with previous PM data which showed that on average AHI PM was 10% lower than AHI PSG. The cases most likely to be missed (false negatives) are those with mild OSA, since the inherent underestimation of the PM may re-categorise mild cases below the diagnostic threshold for OSA (AHI < 5 events/h). False negative rates can be as high as 17% in unattended PM
studies, leading to the recommendation that PMs be used to ‘rule-in’ OSA in the setting of a high pre-test probability.

For laboratory-based PSG it is generally recognised that a first night effect results in poor sleep efficiency and underestimation of OSA\(^{130,450,451}\) due to the large number of sensors applied limiting sleep to the supine posture in an unfamiliar environment\(^{450-452}\). Since most studies examining night to night variability have investigated laboratory PSG, first night effect may well account for some of this variation. Part of the intuitive appeal for home PM relates to the notion of better quality sleep at home and a potentially more accurate diagnosis. Our results for the same individuals over different nights show low variability in PM performance, even though one study was conducted in the laboratory simultaneous with PSG (Figure 6.3). Some studies (using laboratory-PSG) have suggested that the variability is inversely proportional to the severity of OSA, with more severe OSA being more reliably diagnosed\(^{451,453-455}\). However, most PM studies report no night-night change for OSA severity at a group level (mean AHI PSG/AHI PM) but note that misclassification can occur, with the degree dependent upon the cut-off used to define ‘disease’\(^{456-458}\). Two well-designed studies using home PM results reported no bias between nights, first night effect or directional trend, suggesting that PM studies may minimise some of the variability of laboratory PSG\(^{130,458}\). Our results are consistent with most other work indicating minimal or no first night effect when using PMs\(^{130,458}\).
We found no evidence of an order effect \( p = 0.33 \). We hypothesised that subjects studied with laboratory PSG first may have had reduced sleep efficiency and consequent lower severity of OSA. Analysis of misclassifications showed more missed cases \( (\text{AHI} \geq 5 \text{ events/h}) \) in the PSG first group (24%) compared with the PM first group (11%), but these differences were not statistically significant \( (p = 0.08) \). The greater number of cases with mild OSA in the PSG first group increased the likelihood of misclassification. Other studies reporting misclassification have suggested that this is more prominent when a lower AHI cut-off is chosen to ‘rule-in’ disease, providing support for use of PM diagnosis for case selection where pre-test probability is high.\textsuperscript{451,458}

6.6.1 **Strengths and Weaknesses**

The largest barrier to the wide acceptance of PM diagnosis of OSA has been the lack of high quality, adequately powered research studies to strengthen the evidence base.\textsuperscript{128} A grading strategy recommended in the 2003 PM systematic review\textsuperscript{43} was further refined in 2011\textsuperscript{45} to give clear guidance for grading evidence level\textsuperscript{435} and quality rating.\textsuperscript{45} According to this current scheme, our study ranked at level 1b with two quality indicators not met. Data loss for the present PM study of 10.4% from patient-related failures and 3.7% from technical failures compares favourably to previous studies, with a recent meta-analysis reporting an overall 14.6% of poor recordings.\textsuperscript{449} In a recent targeted case finding study in the primary care setting (using the same PM), 7% technical failure was reported.\textsuperscript{459} Consistent with our findings, the most common reasons cited for data loss were patient related issues and partial or complete absence of data.
A good quality study should have a high (> 90%) percentage of patients initially enrolled in the study completing it. Our percentage of patients completing the study was 75% which, while lower than the desired benchmark, is perhaps more realistic given the heavy reliance upon voluntary patient compliance to complete the demanding full study protocol. Our results are comparable with those of three prior studies with a similar design (75%, 65% and 65% respectively). Early in data collection it was clear that the Group 1 subjects were prone to ‘study fatigue’ since many failed to complete the final PM study (P3) at home despite encouragement. This phenomenon may help explain the paucity of adequately powered validation studies to date.

An advantage of our methodology was use of the same analysis platform to score the laboratory PSG and PM recordings by uploading the latter (via EDF) into our laboratory analysis system. Thus the same analytical tools were available for both PM and PSG data and minimised differences in signal interpretation.

Clinical guidelines have repeatedly made clear statements about the importance of manual data review of sleep studies based upon the premise that manual scoring is superior to automated analysis. Many studies have used the PM auto-analysis to determine their results, which appears an obvious inadequacy given the known high misclassification rate for unattended PM studies. Our study sought to minimise such misclassifications by application of standardised manual data review using the same computer platform for both PM and PSG data. We found without exception that manual analysis resulted in fewer missed cases, with percentage reductions ranging from 4% (AHI ≥ 5 events/h) to 18%
6: A COMPREHENSIVE EVALUATION OF A TWO-CHANNEL PORTABLE MONITOR TO ‘RULE-IN’ OBSTRUCTIVE SLEEP APNOEA

(AHI ≥ 30 events/h) (Table 6.4). Unfortunately, our study was not powered to assess the difference in accuracy between auto-analysis and manual scoring and this difference did not reach statistical significance. However, our results are consistent with those of a recent validation study (ApneaLink Ox™) which demonstrated that manual scoring was superior to automatic scoring to ‘rule-in’ OSA. The improved accuracy of manual scoring is of high clinical importance since the goal of the diagnostic test is to optimise case finding.

An acknowledged limitation of PM studies is the potential for misclassification of cases. Our study confirmed that cases with mild OSA can be missed with a PM study. It is important to adhere to a clear clinical pathway such that subjects with a high pre-test probability of OSA but a negative PM test result have a follow up PSG. Further limitations of Type 4 devices such as the inability to identify body position and central apnoea events should be considered when addressing the appropriate PM to incorporate into a clinical service.

Our study addresses a gap in the literature as, to our knowledge, there has not been a validation study of a two-channel Type 4 PM to date that has fulfilled all current recommended study quality criteria and met device validation guidelines. In the most recent review of PMs, those with a minimum of two channels (including oximetry) were graded and evaluated according to a standard set of criteria. It was stated that the strongest evidence for a PM is when tested concurrently with laboratory PSG and in the unattended home setting. Of eleven studies conducted in the recommended setting, only one study (Type 3 device) met the defined criteria to rule in OSA (AHI ≥ 5 events/h) with 95% confidence. Based
on the strength of evidence from the study, in 2013 the AASM gave approval for the manufacturer to use this Type 3 device in the unattended setting in the US.^60 Of the ten prior validation studies of the PM used in this study, three were conducted in the recommended settings of laboratory and home.^437,440,446 Comparison of these results (Table 6.5) revealed a consistent loss of diagnostic accuracy in the home setting relative to laboratory PSG, reinforcing the view that a PM must be validated in the setting of its intended use.

6.6.2 Clinical relevance

Our results are directly applicable to a clinic population with a high pre-test probability of OSA. Indeed, the experimental design simulated clinical use within our sleep service with the degree and detail of instruction given to patients in the study, exactly as intended in a standard clinical setting. We have confirmed that the PM can accurately ‘rule-in’ OSA at home in patients with a high pre-test probability of disease. Our clinic population has a high prevalence (94%) of OSA, hence a LR+ of 1.8 would suffice to ‘rule-in’ OSA and produce a post-test probability of over 95%. It is important that this device is used with an appreciation of the pre-test probability of the target patient group. Providing this is 80% or more then the device will be adequate to ‘rule-in’ disease.^45 However, where the PM study result is negative, the clinical diagnostic pathway must include PSG to minimise missed cases of mild disease.^116 The most recent report on research priorities for ambulatory management of OSA concluded that more high quality evidence was required to support the use of PMs within current practice.^128 More work is needed to address the limitations of PM studies such as
misclassification and the cost of repeat studies. Our study highlights the potential to use Type 4 devices in incorporating ambulatory management into practice alongside PSG, and validates the performance of Type 4 devices as meeting currently recommended guidelines. The inclusion of a PM diagnostic pathway within a busy clinical service may reduce the need for in-laboratory PSG and facilitate more efficient use of healthcare resources.

6.6.3 Conclusion

This study illustrates the utility of a simple diagnostic device in confirming the diagnosis of OSA where it is suspected on clinical grounds in the setting of a high pre-test probability population. Such devices have the potential to facilitate the expeditious diagnosis and treatment of OSA, an under-diagnosed condition with substantial associated morbidity. There is an urgent need for alternative approaches to the diagnosis of OSA due to the limited availability of PSG facilities relative to the prevalence of the condition. While keeping in mind the limitations of ambulatory management of OSA, Type 4 PMs have an important potential role in addressing this gap, and manual data review will ensure optimal case finding.


7 DISCUSSION AND CONCLUSIONS

7.1 OVERVIEW OF FINDINGS

7.1.1 Summary of findings

The research described in this thesis resulted in a number of findings of relevance to the field of sleep medicine. These were;

- Confirmation that sleep clinic patients with untreated OSA have significantly more motor vehicle crashes (MVCs) than the general population (Chapter 3). The mean crash rate ratio (3.07) for OSA patients fell within the range reported in the most recent published meta-analysis, which found OSA associated with a 1.21 to 4.89 increase in mean crash rate ratio.\textsuperscript{16}

- Identification of excessive daytime sleepiness as the primary MVC risk factor in OSA patients. I found a strong association between excessive daytime sleepiness and increased report of near-misses, suggesting that it is those patients with increased sleepiness regardless of OSA severity that are most at risk for MVC (Chapter 3).

- First description of the association of known variants in circadian rhythm genes (Period3) with excessive sleepiness in patients with OSA (Chapter 5).

- Identification of novel associations between three genetic loci in the Period3 gene and risk of dozing (Chapter 5). The observed association was mediated by an interaction between PER3 genotype and severity of OSA (AHI index), a surrogate measure of sleep disruption. Risk of dozing was
genotype-dependent and varied according to AHI level. Patients who were homozygous for the minor allele of rs697693 are at risk of dozing for less severe OSA (a lower AHI of 24) than either major homozygotes or heterozygotes.

- Development of an epidemiological model for the genetic association analysis that found age, gender, obesity, AHI, hypoxia indices and caffeine and alcohol use were associated with both increased Epworth Sleepiness Score (ESS) and a higher risk of dozing in an alerting situation. Genetic associations were modulated by an interaction with AHI but not hypoxia variables. This result suggests that sleep disruption is a greater contributor to excessive sleepiness and risk of involuntarily falling asleep than hypoxia (Chapter 5).

- Comprehensive validation of a Type 4 portable monitor (PM) against the reference standard laboratory-based PSG that addressed many documented limitations of past studies (Chapter 6). I found that the PM was able to ‘rule-in’ OSA in a high pre-test probability sleep clinic population. My study extends previous research in that I have validated the performance of a Type 4 device (two channels) as meeting the currently recommended guidelines.116 My study contributes to the evidence base lending support to the incorporation of ambulatory management of OSA into clinical practice alongside PSG.461 (Chapter 6)
7.2 DISCUSSION

The studies described in this thesis investigate the inter-relationships between OSA, excessive daytime sleepiness and driving risk. Specifically, the potential contributions of genetic, physiological and environmental risk factors to crash risk in OSA were assessed. Two of the studies explored risk factors that may predict increased crash risk in OSA, and found: (i) that untreated OSA is associated with increased crash risk and that there is a strong association between excessive daytime sleepiness and increased report of near-misses (Chapter 3); and, (ii) that three genetic loci involved in control of circadian rhythm are related to sleepiness symptomatology in OSA (Chapter 5). The third study validated a simple home portable monitor to expedite diagnosis and treatment of at-risk OSA cases, especially those with excessive daytime sleepiness (Chapter 6).

Sleep quality may be impaired in OSA due to sleep fragmentation, sleep loss, intermittent hypoxia, or the combined effect of these factors. This derangement of sleep structure contributes to the symptoms of daytime sleepiness and impaired cognition and confers an increased risk of driving and work-related accidents. However there is substantial heterogeneity in the neurobehavioural responses seen among OSA patients that is not readily explained by commonly used clinical measures of severity (e.g. apnoea hypopnoea index), hypoxaemia, or frequency of arousals. Many patients report no daytime sleepiness, or MVCs, while others with mild disease can be severely affected symptomatically. This variability in clinical presentation represents a significant challenge for sleep physicians with respect to risk management for driving. It poses the question: how do we identify those OSA patients at highest risk of accidents?
7.2.1 Driving risk and OSA

One of the greatest hazards associated with untreated OSA is that of increased risk of MVCs due to the frequently associated symptoms of excessive daytime sleepiness and cognitive impairment. The most recent systematic review of OSA and MVC risk found that drivers with OSA have a two to three times increased risk of crash. A meta-analysis was conducted to investigate disease-related factors that may predict increased risk. Unfortunately prediction of risk remains imprecise. Although the disease-related factors of body mass index, apnoea hypopnoea index (AHI), hypoxaemia, and daytime sleepiness are associated with crash risk, the evidence was not found to be conclusive due to limitations in research designs. The work presented in Chapter 3 was designed to address many of the limitations of past crash risk studies for OSA.

My study utilized a comprehensive dataset from an established research resource known as the Western Australian Sleep Health Study (WASHS), consisting of a large clinic-based case-series of OSA patients. I confirmed that patients with untreated OSA reported crashes at a rate significantly (three times) higher than the general community (Chapter 3). This result is consistent with the relative risk reported in three past reviews of OSA and crash risk in both clinical cohorts of OSA patients and community-based samples of drivers. Thus evidence from my study supports the hypothesis that untreated patients with OSA have increased crash risk. In keeping with current Australian Fitness to Drive Guidelines, patients should be advised to avoid or limit driving if sleepy, and should not drive if at high risk (i.e. have a history of frequent sleepiness while driving or have had a drowsy driving crash). However, not all patients with OSA
are sleepy or have crashes, and as many as two thirds may never have a collision. How then are those at greatest risk to be identified?

The most recent systematic review in the US was commissioned to assist with the development of guidelines for commercial vehicle drivers. The prevalence of OSA is high among occupational drivers (ranging from 11.5 to 60%), and research has focussed on this group due to the high cost, fatality rates, and public health impact of truck and bus crashes. The focus of the meta-analysis was to define factors to enhance prediction of risk for occupational drivers, improving the ability of clinicians to differentiate between low- and high-risk individuals for fitness to drive. The work presented in Chapter 3 found a strong association between sleepiness and increased rate of reported near-misses in men and women, and an increased risk of crashes in very sleepy men. These results agree with three past studies. It has long been understood by sleep physicians that many impaired patients minimise their sleepiness-related symptoms at presentation. Symptom minimisation may be prompted by fear of the consequences for their driving licence. However, it is also possible that they have poor perception of these symptoms because of a loss of the normal frame of reference for alertness due to the prolonged nature of their excessive sleepiness. Thus the self-report tool of ESS may be unreliable when licensing is involved. This problem was highlighted in a recent large study of employer-mandated OSA screening and diagnosis in commercial drivers in the US. Online screening followed by PSG for high-risk drivers found that 21% of drivers may have had OSA, but the ESS was not discriminatory between the negative and positive OSA groups. An example of great concern was an individual with extremely severe
OSA (AHI = 164) who reported an ESS score of only two. Thus the subjective tool frequently used by the clinician to assist with the identification of individuals at most crash risk prior to diagnosis is not always helpful. The authors concluded that the ESS was not useful in this group (commercial drivers) as sleepiness was systematically under-reported.⁴⁶³

In a clinical situation where a patient presents with excessive sleepiness and a positive OSA diagnosis, my data suggest that crash risk is increased (Chapter 3). However, even though about 80% of OSA patients complain of both excessive sleepiness and cognitive impairments, some are not sleepy.⁴⁰⁴ Other disease-related risk factors of obesity, hypoxaemia and severity of disease have been investigated, but positive associations have not been consistent. My study found no association between obesity, hypoxaemia or severity of disease and increased crash risk in OSA patients (Chapter 3). A recent Swedish study also found that disease severity was not related to crash risk within the OSA group, and concluded that AHI does not represent a useful index to measure OSA-related cognitive impairment in relation to crash risk.⁴⁷² Past research has found neither OSA severity (AHI index) nor excessive sleepiness (ESS) are consistently associated with crash risk in a dose-response fashion. My work found an association between ESS (subjective report) and self-reported near-misses (Chapter 3). There was a clear trend of increasing near-miss rate with increased sleepiness score in both men and women (Figure 3.3). However the dose effect across sleepiness quartiles was only evident in sleepy men (ESS ≥ 10) in multivariable regression analysis. Some studies have found an association between crash risk and either severe OSA (AHI ≥ 30)¹⁶ or severe EDS (ESS ≥ 16),²⁷² but not consistently so. In addition,
severity of OSA is not correlated with sleepiness. Thus, there are limits to the accuracy of reported sleepiness in predicting crash risk, especially in the occupational driver setting. The wide variation in the degree of sleepiness among patients with severe OSA\textsuperscript{36,149} has led to increased research into other measures of cognitive impairment that may better predict crash risk. In addition to chronic daytime sleepiness, the hallmark features of cognitive and daytime dysfunction in OSA are mood and cognition changes, impaired vigilance and driving difficulties.\textsuperscript{20} Impaired vigilance and excessive sleepiness are most likely to impact on safety-sensitive tasks such as driving, and recent research has focussed on these domains.

A recent Swedish study has explored other facets of cognitive dysfunction (lapses and reaction times) in OSA patients for potential predictors of crash risk, and found that attention deficits detected in neurocognitive tests differentiate between OSA patients with or without a MVC history.\textsuperscript{464} Two standard neurocognitive tests were performed during the day after a sleep study and more frequent lapses and longer mean reaction time in OSA patients with a positive MVC history was demonstrated. These findings suggest that vigilance is reduced over time in such patients, which might be attributable to microsleeps, distraction or to increased global cognitive impairment.\textsuperscript{464,465} An acknowledged limitation of this study\textsuperscript{464} was low sample size, however strengths included the use of objective measures of both cognitive dysfunction and registry defined MVC history in OSA patients. Karimi et al. contribute a useful idea for the development of an objective measure predicting OSA-related crash risk, thus avoiding the problem of under-report of sleepiness symptoms.
Some individuals cope with sleep loss more readily than others. Total sleep deprivation has been widely studied in an attempt to understand the performance impairments associated with sleep loss. These findings are directly applicable to understanding shift work effects. More recently sleep restriction has become an increasingly pervasive problem in the community, raising concerns about the increased risk of fatigue related errors, injury, and accidents. The high incidence of fragmenting sleep disorders, especially OSA, has also drawn attention to the effects of sleep disruption. Studies of sleep deprivation (total and partial), and sleep fragmentation have found that all have significant detrimental effects on cognitive performance and sleepiness. Several findings of work in healthy individuals are pertinent to understanding the neurobehavioural deficits of sleep deprivation in OSA patients. Neurobehavioural deficits from sleep loss varied significantly among individuals, but were stable within individuals. These differences were not accounted for by sleep history, and the differences were postulated to constitute a differential vulnerability ‘trait’ to sleep loss. The variability clustered on three distinct neurobehavioural dimensions: self-evaluation of sleepiness, fatigue, and mood; cognitive processing capability; and behavioural alertness measured by sustained attention performance. This trait-like differential vulnerability to sleep loss is likely to be determined in part by genetic polymorphisms as summarized in the following Section 7.2.2.
The variability in response between the dimensions described by Van Dongen and Dinges\textsuperscript{23} warrants further consideration since impairments are stable within individuals but not directly related, such that impairment in one domain is not correlated with impairment in another. Decrements in subjective sleepiness and mood varied among individuals, independent of impairment in objective measures such as sustained attention. For example, an individual with a low sleepiness score (‘resilient’ to sleepiness) may perform very poorly on the sustained attention test (‘vulnerable’ to lapses and errors), thus demonstrating resistance to impairment on one outcome measure, and vulnerability to impairment on another. This lack of relationship between subjective and objective measures of neurobehavioural impairment has implications for safety and accident risk, and for the clinical assessment of sleepiness. A sleep-restricted individual (such as a shift-worker or an OSA patient) who does not feel sleepy while working or driving may nonetheless have increased attentional lapses or physiological sleepiness, and so be at increased risk of an accident. It seems that resistance or vulnerability to sleep loss is multidimensional, but neurobiological correlates that define this phenotype or trait have not yet been reported.

The observed differential vulnerability to sleep loss in healthy sleep deprived individuals may help explain the poor relationship between severity of OSA and daytime impairment. There are clear parallels between the neurobehavioural deficits found in sleep-deprived healthy individuals and in sleep disrupted OSA patients.\textsuperscript{191} The cognitive domains affected are similar, and two Australian studies have explored the hypothesis that untreated OSA patients would be more vulnerable to sleep deprivation than healthy controls, due to the additional
neurobiological ‘load’ of sleep fragmentation. Both studies measured reaction time, simulated driving and self-reported sleepiness to explore increased driving risk factors. Wong et al. found no significant differences between OSA cases and controls using a 40 hour sleep deprivation protocol, with the same decrements in cognitive function across 24 hours. Vakulin et al. evaluated the effects of both sleep restriction and low dose alcohol on simulated driving in OSA cases and controls. They differentiated resistant (60%) and vulnerable (40%) cases, but could not predict membership of either category on the basis of any OSA-related measures. However, both studies found significant inter-individual variability in vulnerability to sleep deprivation confirming Van Dongen’s findings in healthy individuals. Thus, there is evidence in OSA cases that differential vulnerability to sleep loss may be mediated in part by genetic factors.

7.2.2 Genetic variants associated with risk of dozing

The genetic component of this thesis (Chapter 5) aimed to investigate whether genetic variants known to regulate circadian rhythm and sleep homeostasis were also associated with excessive sleepiness in patients with OSA. The study found a novel association between three PER3 variants and increased risk of dozing in a low somnificity (alerting) situation. The relationship was mediated via an interaction between PER3 genotype and severity of OSA (AHI index), a measure of sleep disruption. It could be speculated that PER3 genotype variation contributes in part to the differential vulnerability to excessive daytime sleepiness in patients with OSA despite their having a similar severity of disease. To my knowledge this is the first study to report an association between Period 3 genotype and excessive sleepiness in a well-phenotyped cohort of OSA patients.
In 2007 a genetic association with a sleepiness phenotype was first reported. A GWAS of sleep and circadian phenotypes confirmed significant heritability of sleepiness, usual bedtime and usual sleep duration using linkage analysis and association tests identified a genetic variant on chromosome 5 (PDE4D) as a possible mediator of sleepiness. However the association for PDE4D was not replicated in subsequent studies. With respect to Period genes, most work has focussed on PER2 and PER3 since candidate gene studies found associations between these variants and both sleep phase disorders and chronotype. A PER3 variable-number tandem-repeat (VNTR) with 4 or 5 repeats is associated with delayed sleep phase syndrome and diurnal preference. This finding marked the beginning of a series of investigations devoted to understanding the relationship between this PER3 VNTR variant and individual differences in sleep-wake regulation. Population studies have shown that among those of European-ancestry, 10% of individuals are minor allele homozygotes (PER3^5/5) and, about 50% are major allele homozygotes (PER3^4/4). This relatively high allele frequency and the established association with a sleep/circadian phenotype has provided a useful model for the prospective study of the impact of the Period 3 gene on sleep-wake regulation. Viola et al. used a sleep deprivation protocol to investigate whether genotype-dependent differences conferred individual vulnerability or resistance to sleep loss. They found that minor homozygotes (PER3^5/5) had a significantly different response to sleep deprivation, with much greater cognitive deficits. This was the first study to suggest that this PER3 polymorphism may be an important marker for individual differences in sleep and susceptibility to sleep loss. A series of studies has explored the effects of both total and partial sleep deprivation on healthy subjects recruited by PER3 genotype. Overall, PER3 minor homozygotes
(PER3<sup>5/5</sup>) show functional deficits and increased sleepiness in response to total sleep deprivation, compared with PER3 major homozygotes (PER3<sup>4/4</sup>). The results have been less convincing for partial sleep deprivation but there are clear differences between the two PER3 homozygotes. A recent study examined individual differences in sleepiness and circadian phase in night shift workers, comparing 4-repeat and 5-repeat carriers.<sup>367</sup> The group found significantly higher sleepiness and maladaptive circadian phase in the 5-repeat workers, concluding that they may be at greater risk for occupational and driving accidents. In summary, a large body of work has found that the PER3 VNTR is a genetic marker for vulnerability to the effects of extensive sleep deprivation and circadian misalignment.<sup>31</sup>

My study in Chapter 5 describes genetic association analyses between SNPs in the PER3 gene and the outcome of excessive sleepiness in OSA patients. To my knowledge this association analysis has not been explored previously. I postulated that some individuals with OSA may be vulnerable to the sleep disruption associated with OSA due to the presence of particular Period gene polymorphisms. This vulnerability to sleep debt mediated in part by genetic polymorphisms could contribute to the symptom of excessive sleepiness and cognitive impairment in untreated OSA patients, and thereby confer an increased risk of driving and work-related accidents. There was an association between three PER3 SNPs and risk of dozing in a low somnificity situation. The low somnificity phenotype identifies individuals at risk of falling asleep in an alerting situation and potentially at risk of dozing in a safety-critical situation.
Within the somnificity phenotype, the positive association suggests an interaction effect between \textit{PER3} genotype and risk of dozing in individuals, mediated by severity of OSA. It may be speculated that the sleep disruption of OSA is a greater contributor to daytime sleepiness than hypoxia. This finding is aligned with past work examining daytime sleepiness in OSA which concluded that sleep fragmentation was the primary contributor to daytime sleepiness, and that intermittent hypoxia was related to cognitive impairment\textsuperscript{302} However, the authors noted that sleep fragmentation and intermittent hypoxia are inter-related in their effects, and that the extent of inter-correlation among variables representing sleep fragmentation and intermittent hypoxia is not yet understood. A recent meta-review of neurocognitive function in OSA concluded that the severity of sleep fragmentation appeared to be linked to deficits in attention/vigilance, while impairments in global cognitive function appear linked to hypoxia\textsuperscript{465} Thus both pathophysiological abnormalities contribute to neurocognitive dysfunction in OSA, but sleep fragmentation due to OSA-related arousals seems to contribute more to daytime sleepiness than hypoxia. My findings (Chapter 5) support the conclusion that sleep disruption in OSA contributes to the risk of dozing more so than hypoxia.

It is likely that there are person-specific aspects of sleepiness comprised of both sleep and non-sleep factors that combine to create the ‘sleepiness’ phenotype for an individual. Sleep physicians and researchers have come to understand that an individualised approach to MVC risk assessment in OSA patients is required. In conjunction with parameters related to OSA, consideration must be given to all factors that influence crash risk, such as sleep duration and circadian effects in shift workers, age, gender, driving exposure (kilometres driven), and sensitivity to
stimulant and sedative compounds. There has been considerable interest in the
determination of biomarkers of individual differences to sleep loss, both
behavioural and genetic. Identification of individuals vulnerable to sleep loss
would assist with management of people at risk in safety-critical settings. The
study described in Chapter 5 explored the concept that variability in sleepiness
symptomatology may be related in part to genotype-dependent differences.

The sleepiness phenotype in both sleep-restricted (but otherwise healthy)
and sleep-disrupted (OSA-related) individuals is a complex trait. Complex traits
are influenced by the contribution of many genetic and environmental
determinants, and their interactions. The sleep phenotype for an individual needs
to take account of age, gender, short or long sleep requirement, morning/evening
typology, and sleep propensity across 24 hours. Individual differences in need for
sleep are likely underpinned by genetic variants for each sleep phenotype
component, combined with environmental effects. A recent twin study has shown
substantial heritability of vulnerability to sleepiness (83%) and that heritability is
mediated by PER3 and adenosine deaminase (ADA) genotype.369 Recent consortia
collaborations for complex traits such as height and weight have found that the
effect sizes of associated genetic variants are very small. As such, the authors
concluded that there are important methodological issues regarding careful
phenotyping and the large sample sizes needed to investigate complex traits
because of the polygenic nature of phenotypic traits and the small effect sizes of
each variant contributing to the phenotype.378 Thus the challenge remains to
elucidate the complex biology that underlies the sleepiness trait in OSA. From a
clinical perspective, predicting accident risk in a patient at initial presentation
remains a challenge. Consideration should be given to the multiple factors related to disease, sleep, environment, social situation, motivation and perception that interact to create an individual sleep phenotype.

7.2.3 **Validation of a home sleep monitor to expedite diagnosis of sleepy individuals**

The burden of undiagnosed OSA in the community is high, and as such, there is a need for validated cost-effective diagnostic tools to expedite triage of suspected OSA cases, especially those who are very sleepy and at risk of accidents. The primary aim of the validation study described in Chapter 6 was to evaluate a simple home sleep monitor as a triaging tool for suspected OSA in a population referred to a specialist sleep clinic. Although portable sleep technology has been embraced by many countries, and practice parameters were first published over twenty years ago, there has been reluctance by professional associations representing sleep medicine to endorse the use of more simple devices (1-7 channels). However simple Type 4 devices in particular (1-2 channels) have the advantages of being reliably self-applied by the patient at home, allowing sleep to occur in the familiar home environment, more ready availability and a substantially lower cost than overnight laboratory PSG.

My study (Chapter 6) has comprehensively validated a Type 4 portable sleep monitor (PM) against the reference standard laboratory-based PSG. There has been a paucity of well-conducted validation studies of Type 4 PMs, which has hampered their incorporation into ambulatory clinical pathways for the management of OSA. My study addressed many of the limitations of past studies, verifying that the device could rule-in OSA in a high pre-test probability sleep clinic.
population. This validation lends support to the incorporation of an ambulatory management pathway into practice alongside PSG. It provides a tool to triage cases of suspected OSA when a clinician is especially concerned about high risk of accidents, such as in occupational drivers or mine-site workers.

Obstructive sleep apnoea is increasingly common, with recent population prevalence estimates from the Wisconsin cohort study ranging from 9 to 17% of middle-aged men and women. This recent estimate represents a disturbing increase in prevalence over the past two decades, with ageing of the population and increasing prevalence of obesity likely driving influences. Similar estimates have been reported for moderate to severe OSA in middle-aged general population samples in Australia (9.1%), Switzerland (23.4%) and Iceland (20.0%). In parallel with the increasing prevalence of OSA, the incidence of fatal driving crashes is increasing, prompting the WHO to declare a decade of driving safety (2011 to 2020) in order to halt or reverse this trend. A recent review reported that a large proportion of driving crashes around the world are related to inadequate or disordered sleep, with OSA being the most prevalent of sleep disorders. A substantial proportion (16-20%) of serious highway crashes have been linked to fatigue, and OSA is highly prevalent in commercial drivers. Thus there is an imperative internationally to address the large undiagnosed OSA population among drivers, and so ameliorate crash risk.

Arguments for this have been made from a public health perspective since economic analyses have shown that OSA-related driving crash costs are very high, and the net savings of diagnosis and treatment far outweigh the costs. In both Europe and the US, significant resources have been directed toward validation of
Ambulatory diagnosis and therapy. A European directive regarding OSA testing in drivers was scheduled for implementation in December 2015. The key objective is to encourage drivers with OSA to seek diagnosis and treatment and not inhibit them coming forward. In the US there has been particular emphasis directed toward management of OSA in commercial drivers. Many transportation companies have implemented employer mandated OSA screening and diagnosis in commercial drivers, using ambulatory testing as a part of this process. Studies to date have found that after treatment, monthly medical costs decreased by 50%, reported driving crashes reduced 74%, and driver retention rates increased 2.3-fold. These data point to substantial benefits for employer, employee and the community generally.

Ambulatory testing for OSA represents a way forward in screening in high-risk occupations, and as an objective assessment of large populations for OSA. I validated a Type 4 portable sleep monitor to rule-in OSA in a clinical population with a high pre-test probability of OSA, allowing ambulatory diagnosis to now be incorporated into clinical and epidemiological investigations with confidence.

7.3 CLINICAL IMPLICATIONS AND FUTURE CONTEXTS

My study has confirmed that while patients with untreated OSA are at generally increased risk of motor vehicle crash, risk is highest in those who are excessively sleepy. Past research has found increased crash risk in association with untreated OSA in both clinical and population samples, independent of daytime sleepiness. Drivers do not always perceive impairment, and thus may continue to drive when sleepy putting the lives of others at risk. The concept of somnificity described in Chapters 4 and 5 may provide a useful preliminary tool to detect a
sub-group of vulnerable drivers at initial consultation. Where patients concede a risk of dozing in an alerting situation, it may be possible to use a positive response to highly specific questions (i.e. 6 and 8) of the ESS (Table 1.2) as a marker of elevated risk, particularly where the total sleepiness score is normal (i.e. total ESS score < 10). A significant association between abnormal sleepiness in a motor vehicle and self-reported crash involvement has previously been shown in occupational drivers by applying this concept. A simple low somnificity tool could be easily applied to flag at risk drivers, at least until such time as reliable biomarkers or vigilance tools become available.

Clinicians should also be aware that the neurocognitive domains most sensitive to sleep loss do not overlap, i.e. a low ESS score for an individual does not predict good performance on a sustained attention task. Thus although a person may not be subjectively sleepy, their performance on performance vigilance testing can be impaired, increasing driving risk. This inter-individual variability in response to sleep loss was first demonstrated in studies of healthy individuals, but subsequent work in OSA patients has confirmed this same variability in response to further sleep deprivation. The results of my genetic association analysis in Chapter 5 support the notion of a genetic basis to vulnerability to or resilience from sleep loss. Novel PER3 genetic loci linked to sleepy phenotype in OSA patients may represent potential genetic markers of vulnerability to sleep loss. Great progress in our understanding of genotype-dependent vulnerability to sleep loss has been made over the past five years using a prospective approach whereby subjects are recruited by genotype. The relatively high genotype frequencies for the top three SNPs in our study (5%, 19% and 20% respectively) would allow
prospective recruitment of OSA patients to study cognitive performance in response to sleep deprivation with adequate power. This may help elucidate the role of genetic variants interacting with OSA-related variables to promote excessive sleepiness symptoms. These genetic biomarkers may identify those who are particularly susceptible to the effects of sleep loss, and thereby at increased risk of sleepiness-related accidents.

My study (Chapter 3) found a strong relationship between excessive sleepiness and near-misses extending existing knowledge since the most recently published meta-analysis found no substantial association between ESS scores and crash risk in drivers with OSA.\(^{16}\) The study was well-powered statistically but was based upon self-report near-misses and crashes. Accuracy would be improved in future by the use of objective crash records. In addition to the dataset used in this study, the WASHS has a large retrospective dataset (about 30,000 records) consisting of longitudinal data for patients who underwent sleep studies from October 1988 to April 2010. The majority had laboratory PSG and were diagnosed with OSA (91%). In Western Australia databases held by both the Department of Transport (DOT) and Main Roads are available for crash research projects. A future comprehensive linked data study linking the WASHS records and those of a matched set of controls to the objective crash records at DOT and Main Roads would provide a valuable extension of our work.
7.4 LIMITATIONS

There are several limitations to the crash risk study reported in Chapter 3. There is potential recall bias with respect to self-reported crashes. The crash questions were asked as part of a sleep health questionnaire and not in relation to occupation, but non-response for near-miss and crash questions was a minor issue. Nevertheless non-response would bias results towards the null hypothesis (i.e. that there is no relation between OSA and crashes) and thus strengthens the positive findings of our study. The self-reported crash data was for the entire driving history for the cohort and so may be subject to some recall or report bias. Non-response for crash questions is an example of report bias. There may be measurement bias associated with the use of police-reported crashes compared with self-report for our cohort. Future studies would benefit from use of objective crash data records, as suggested earlier. Most data collected to investigate OSA and crashes is derived from sleep clinic populations and thus not necessarily applicable to community-based populations. However, data from Young et al. (Wisconsin cohort study) for a community sample free of clinic bias were comparable in magnitude to clinic-based studies, supporting the generalizability of our findings. Gender differences found in our study may be confounded by driving exposure, which we did not measure. More men than women are occupational drivers,\textsuperscript{148} giving higher driving exposure and increased crash risk. Finally, a subjective measure of sleepiness was used (ESS). Although validated for use in both clinical and research domains, subjective measures such as ESS are not as reliable as objective measures, particularly in the commercial driving domain.
The findings of my genetic analysis (Chapter 5) were limited by moderate sample size: 50 loci were analysed in 1,053 cases. Although a relatively large sample size compared to previous published genetic studies, my sample size was inadequate to detect the small effect sizes anticipated for a genetically complex trait. It will be important to replicate results for our significant PER3 SNPs in independent larger samples. The numbers in the minor homozygote group (for rs697693) in particular were low (n = 56). The data used in my analysis were generated from a larger GWAS on a sub-set of the WASHS cohort that was stratified by BMI. A GWAS conducted on the complete WASHS cohort may strengthen our findings. The timing of the study meant that the PER3 VNTR SNP (a marker of vulnerability to sleep loss) was not selected for genotyping, and this represents an opportunity for future work. The population sample studied was predominantly of European-ancestry (representative of the community in Western Australia), so that results are not generalizable to other ethnicities. Finally, a general limitation (found in most genetic association studies of sleep phenotypes to date) was the reliance on questionnaire-data (self-report), especially for driving crashes. However, a strength was the phenotyping for sleep variables since all patients had overnight PSG.

My portable sleep monitor validation study (Chapter 6) was carefully designed to address many of the limitations of past validation studies. Nevertheless, the study ranked at level 1b (grading evidence level and quality rating) with two quality indicators not met: data loss should be < 10% and > 90% of enrolled patients should complete their sleep study. Our data loss was 14%, and percentage completed was 75%, both of which compare favourably with studies of
equivalent study design. Finally, our validation was conducted in a sleep clinic population and therefore the results cannot be translated easily to a community population with a different (lower) OSA prevalence.

7.5 CONCLUSION

Careful consideration of the symptom of daytime sleepiness is crucial when assessing accident risk in OSA patients. However, many OSA patients are not excessively sleepy, which presents a dilemma for sleep physicians with respect to assessment of accident risk prior to diagnosis and treatment. This thesis presents data relevant to this relationship between excessive daytime sleepiness and increased driving risk in OSA patients. My research broadens knowledge of the relationship between sleepiness and driving risk in OSA; reports novel associations between circadian rhythm genes and vulnerability to dozing modulated by severity of OSA; and validates a simple method to expedite identification of at risk individuals with OSA.
8 REFERENCES

REFERENCES

35. Vakulin A, D’Rozario AL, Grunstein RR. Driving impairment and accident risk in sleep apnea: We need better assessment tools. *J Sleep Disor: Treat Care* 2012; 1(1).


78. Dijk DJ, Franken P. Interaction of sleep homeostasis and circadian rhythmicity: Dependent or independent systems? In: Kryger M, Roth T, Dement WC, eds. Principles


8: REFERENCES


464. Karimi M, Hedner J, Zou D, Eskandari D, Lundquist L, Grote L. Attention deficits detected in cognitive tests differentiate between sleep apnea patients with or without a motor vehicle accident. *Sleep medicine* 2015; **In press**.