Familial Aggregation of Insomnia in Hong Kong Chinese: Case-control Study in a Prospective Cohort

ZHANG, Jihui

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Submitted by ZHANG Jihui

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ABSTRACT

Backgrounds and Aims: Insomnia is a common sleep problem with significant health burden to individuals, families and society. Several risk factors contributed to the development of insomnia with significant familial aggregation phenomenon. According to this prospective study, we aimed to 1) explore the longitudinal course and outcomes of insomnia in both children and their parents; 2) confirm the familial aggregation and heritability of insomnia by detailed clinical interviews; 3) explore the potential biological markers of insomnia in terms of heart rate variability, 24-hour urinary cortisol and serial salivary cortisol.

Methods:

Phase 1 study: The package of questionnaires for adolescents, their siblings, fathers and mothers are mailed to the 3416 families, among which 1611 families (47.1%) returned. Individuals with difficult initiating sleep (DIS), difficult maintaining sleep (DMS) and/or early morning awakening (EMA) at least thrice/week were defined as insomniacs.

Phase 2 and 3 studies: A total of 236 families were invited into phase 2 study. A case-control study with detailed clinical, sleep, psychiatric and biological assessment were conducted in adolescents with insomnia (n=75) and those adolescents without insomnia (n=161) as well as their first-degree ralatives. The assessments included 1)detailed clinical assessments, including: medical history and structured psychiatric interview; 2) detailed psychiatric assessments; 3) Objective and subjective sleep assessment with 3 days actigraphy and sleep questionnaire; 4）heart rate vairability; 5) 7-time points 1-day salivary cortisol and 6） 24-hour urinary cortisol.

Results:

Phase 1: The prevalences of insomnia were 4.5%, 10.8% and 13.9% at baseline and 6.6%, 8.1% and 11.6% at follow-up for children, fathers and mothers respectively. Similar incidence rate of insomnia was found across adolescents, fathers and mothers (6.2%, 5.4% and 6.8% respectively, $p>0.05$), while highest persistence rate of

insomnia was found in mothers (43.8%), followed by fathers (26.9%) and adolescents (14.9%) (mothers vs adolescents OR(95%CI)=4.43(2.22-8.86); mothers vs fathers OR(95%CI) = 2.11(1.31-3.42); fathers vs adolescents OR(95%CI) = 2.17(0.98-4.52)). Insomnia at baseline was significantly associated with frequent episodes of allergic rhinitis, asthma, and laryngopharygngitis and chronic use of medicine at follow-up in adolescents($p<0.05$). Insomnia at baseline was also significantly associated with poor medical outcomes in adults, including frequent allergic rhinitis, otitis media, hypertension, arthritis, psychiatric disorders, chronic pain and gastroesophageal reflux disease at follow-up in middle-aged adults ($p<0.05$).

Phase 2 study:

The first degree relatives' recurrent rate was higher in those adolescents with insomnia than those adolescents without insomnia (43.9% vs 22.9% for current insomnia and 51.1% vs 28.0% for lifetime insomnia, respectively $p<0.001$). Genetic analysis showed that the heritabilities were 0.57 ± 0.19 for current insomnia and 0.67 ± 0.13 for lifetime insomnia after adjusted for age and gender. There was significant synergistic interaction between parental history of insomnia and life stress on the development of insomnia of offsprings ($p=0.002$). Insomnia disorder and its severity were also found to correlate with neuroticism personality, psychological distress and poor quality of life. The phenotypic correlations of insomnia with these factors could

be mainly explained by genetic component in bivariate genetic analysis.

Phase 3 study: 1) Subjective sleep quantity and quality was consistently and negatively correlated with 24-hour urinary cortisol and salivary cortisol levels in adolescents. However, there was no such association in adults. 2) Adolescents with insomnia diagnosis had lower salivary cortisol at 0 minute after waking up (T1) but less decrease in AUCi3 than non-insomniac adolescents. Althougth there was no difference in serial salivary cortisol between insomniacs and non-insomniacs in adult, insomnia diagnosis interacted with gender on the effects of ACUi and salivary cortisol level at 10:00 pm. 3) There was no difference in 24-hour urinary cortisol between insomniacs and non-insomniacs. 4) There were some inconsistent associations of salivary cortisol with objective and subjective sleep parameters between continuous and dichotomized approaches. Fox example, there was no correlation between salivary cortisol and objective sleep measures in adults when using continuous variables, but, short sleepers as defined by objective TIB$\leq$400 minutes had higher cortisol levels at T1 (13.5$\pm$7.9 nmol/L vs 11.2$\pm$5.0 nmol/L) and T2 (14.0$\pm$6.0 vs 11.5$\pm$6.2 nmol/L) than their counterparts (TIB>400 minutes). In brief, cortisol (both salivary and urinary samples) level was more likely to be correlated with subjective measures of sleep than objective measures or insomnia diagnosis. In particular, the association predomninantly occurred in adolescent group.

Conclusions:

Insomnia is commonly found in both adolescents and adults with moderate persistence rate after 5 years in Hong Kong Chinese. Our findings of increased risk of chronic medical burdens and various upper airway inflammatory diseases in both adolescent and adult subjects with insomnia suggested that insomnia requires comprehensive medical attention. Insomnia is a highly heritable disorder with robust familial aggregations, with a heritability of 0.67 for lifetime insomnia. We found gene-environment interaction on the pathogenesis of insomnia. Our findings strongly suggested the necessity of further molecular genetic analysis on insomnia. Daytime HRV, 24-hour urinary cortisol and serial salivary cortisol might not be the reliable biological markers for insomnia.

香港華人失眠的家族聚集性：基於一個前瞻性隊列的病例-對照研究

背景及目的：失眠是一個常見的睡眠問題並對個人，家庭及社會造成沉重的負擔。我們及其他人的早前研究發現，失眠具有明顯的家族聚集性並與某些危險因素有關。通過本研究，我們試圖：1) 瞭解香港華人兒童和成人的失眠症的長期變化及失眠對慢性身體疾病的影響； 2）通過詳細的臨床會晤確認失眠的家族聚集性及遺傳度； 3）從心率變異度，24 小時尿皮質醇及系列唾液皮質醇方面瞭解與失眠有關的潛在生物學標記。

方法：

第一階段：通過郵寄的方式，我們把問卷散發給 3416 個家庭，合共 1611 個家庭（47.1%）返還問卷。具有睡眠入睡困難，睡眠維持困難及早醒至少每週三次 的個體則被定義爲失眠患者。

第二和第三階段：本研究以青少年作爲先證者。通過詳細的臨床會晤，分別有 75 例青少年被確認爲失眠患者及 161 例被確認爲非失眠患者。他們的一級親屬也進行相類似的評估。這些評估包括：1）詳細的臨床評估， 包括疾病史和結構化精神科會晤；2）詳細的問卷調查； 3）3 天客觀（體動記錄儀）和主觀（睡眠問卷）的睡眠評估； 4）心率變異度； 5）7 時間點的唾液皮質醇濃度及 6）24 小時尿皮質醇總量。

結果：

第一階段：基線失眠的患病率在青少年爲 4.5%，父親爲 10.8% 及母親爲 13.9%；而隨訪中失眠的患病率在青少年爲 6.6%， 父親爲 8.1%及母親爲 11.6%。在不同的組別之間，新發病率相若； 但失眠的持續率在母親組最高（43.8%），其次爲父親組（26.9%）及青少年組（14.9%） （$p<0.05$）。在青少年組，基線的失眠明顯與最近一年頻繁發作的過敏性鼻炎、哮喘和咽喉炎及慢性使用藥物有關（$p<0.05$）。 在成人組，基線的失眠不但明顯與最近一年頻繁發作的過敏性鼻炎、中耳炎、及咽喉炎有關，並且與隨訪期的高血壓、關節炎、精神科疾病、慢性疼痛和胃食管返流有關（$p<0.05$）。

第二階段：失眠青少年的一級親屬的患病率明顯高於非失眠青少年 (現患失眠爲 43.9% vs 22.9%而終身失眠爲 51.1% vs 28.0%, $p<0.001$)。遺傳分析顯示現患失眠的遺傳度爲 0.57 ± 0.19 而終身失眠的遺傳度爲 0.67 ± 0.13 。父母的失眠病史和生活壓力對青少年的失眠發生具有明顯的協同交互作用（$p=0.002$）。失眠及其嚴重程度跟神經質性格、心理學症狀及生活品質差有明顯關係。雙變量遺傳學分析進一步發現：失眠與神經質性格、心理學症狀及生活品質差的關係可主要歸因於遺傳因素。

第三階段： 1) 在青少年中，主觀睡眠時間和品質與 24-小時尿皮質醇和唾液皮質醇（睡前及醒後）呈負相關。但類似的相關在成人中未發現。2）失眠的青少年醒後的唾液皮質醇水準較低但在 AUCi3 的下降明顯低於非失眠青少年。儘管

唾液皮質醇在成人失眠者和非失眠者之間無差異，但失眠症與性別對 ACUi 在夜間（10：00 pm）唾液皮質醇有交互作用。3）在青少年或者成人中，心率變化度和 24 小時尿皮質醇總量在失眠患者和非失眠者之間沒有任何差異。4）儘管某些系列唾液皮質醇濃度指標在失眠者和非失眠者之間有統計學差異，但這些差異在不同年齡組（青少年和成人）和性別之間缺乏一致性。

結論：失眠在香港華人青少年及其父母中常見並且具有適度的持續性。慢性失眠可增加軀體疾病的發病風險的發現提示失眠需要精神科及內科醫師的關注。失眠具有明顯的家族聚集性和高度的遺傳傾向性。本研究同時發現失眠可通過基因和環境交互協同作用而發病。我們的研究提示對失眠的遺傳學進一步進行研究的必要性。心率變異度，24 小時尿皮質醇總量和系列唾液皮質醇濃度很可能不是失眠的可靠生物學指標。

PUBLICATIONS

Papers published or accepted

1. Zhang J, Lam SP, Ho CK, Li AM, Tsoh J, Mok V, Wing YK. Diagnosis of REM sleep behavior disorder by video-polysomnographic study: is one night enough? **Sleep**. 2008 ;31(8):1179-85.
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8. Li SX, Lam SP ,Yu MWM, Zhang J, Wing YK. Nocturnal sleep disturbances as a predictor of suicide attempts among psychiatric outpatients. **Journal of clinical psychiatry.** (in press)

9. Lam SP, Zhang J, Tsoh J, Li SX, Ho CK, Mok V, Chen A, Wing YK. REM sleep behavioral disorder in psychiatric population. **Journal of clinical psychiatry.** (in press)

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1. Zhang J*, Zhang B* (co-first author), Ho CK, Li AM, Lam SP, Wing YK. Objective and subjective "sleepiness" in healthy subjects: an exploratory study for the implication of the concept of "sleepability". **Sleep and Biological Rhythm** (submitted).

2. Zhang J, Ma RCW , Kong APS , So WY, Li AM, Lam SP, Li SX, Yu MWM, Ho CS, Chan MHM, Zhang B, YK Wing. Relationship of sleep quantity and quality with 24-hour urinary catecholamines and salivary awakening cortisol in healthy

middle-aged adults. **Sleep** (submitted).

3. So HK, Li AM, Au CT, Zhang J, Lau J, Fok TF, Wing YK. A population study of night sweating in children: prevalence and risk factors. **Journal of Pediatrics** (submitted).

4. Li SX, Yu MWM, Lam SP, Zhang J, Li AM, Lai KYC, Wing YK. Frequent nightmares in children: familial aggregation and associations with parent-reported behavioral and mood problems. **Sleep** (submitted).

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LIST OF ABBREVIATIONS

The following abbreviations are commonly used in this thesis

5-HTTLPR	Serotonin Transporter Length Polymorphism
95%CI	95% Confidence Interval
ACTH	Adrenocorticotropic Hormone
ASLES	Adolescent Self-reported Life Event Scale
ANCOVA	Analysis of Covariance
AST	Actual Sleep Time
AUC	Area Under the Curve
BDI	Beck Depression Inventory
BMI	Body mass index
CAR	Cortisol Awakening Response
DBAS	Dysfunctional Beliefs and Attitudes About Sleep Scale
DIS	Difficulty Initiating Sleep
DISC-IV	Diagnostic Interview Schedule for Children-Version 4
DMS	Difficulty Maintaining Sleep
DSM	Diagnostic and Statistical Manual of Mental Disorders
EMA	Early Morning Awakening
FFI	Fatal Familial Insomnia
FFT	Fast Fourier transformation
FIRST	Ford Insomnia Response to Stress Test
GEE	Generalized Estimating Equation
GERD	Gastroesophageal Reflux Disease
HADS	Hospital Anxiety and Depression Scale
HF	High Frequency Power
HPA	Hypothalamus-pituitary-adrenal
HRV	Heart Rate Variability
ICD-10	International Statistical Classification of Diseases
ICSD	International Classification of Sleep Disorders
ISI	Insomnia Severity Index
LF	Low Frequency Power
NEO-FFI	NEO Five Factor Inventory
NHANES	National Health and Nutrition Examination Survey
NRS	Non-restorative Sleep
OR	Odds Ratio
PSG	Polysomnogram or Polysomnography

PSQI	Pittsburgh Sleep Quality Index
QoL	Quality of Life
RRSTD	Standard Deviation of all RR intervals
SBP	Systolic BloodPressure
SCID	Semi-structured Clinical Interview for DSM-IV
SE	Sleep Efficiency
SES	Socio-economic Status
SF-36	36-item Short Form Health Survey
SNP	Single Nucleotide Polymorphisms
SNS	Sympathetic Nervous System
SOL	Sleep Onset Latency
SOLAR	Sequential Oligogenic Linkage Analysis Routines
SPSS	Statistical Package for the Social Sciences
SSI-28	28-item Somatic Symptom Inventory
SWS	Sleep Wave Sleep
TIB	Time In Bed
VAS-pain	Visual Analogue Scales for pain
WASO	Wake After Sleep Onset

CHAPTER ONE INTRODUCTION

1.1 Insomnia: implications for pervasive health care burden

Insomnia is the commonest sleep disorder with significant health care burden and morbidities. Insomnia is correlated with pervasive impairments including reduced daytime alertness, fatigue, increased health care utilization, accident risk, reduced productivity and subjective quality of life.[1, 2] Insomnia has been found to increase total costs (direct and indirect) in a rate of around US$1253 in younger adults and US$1253 in elderly when compared with subjects without insomnia in US population respectively.[3] Another Canadian study further confirmed that cost of insomnia was much higher in those subjects with insomnia syndrome (insomnia symptom plus daytime function impairments) than subjects with insomnia symptom and good sleepers (Cdn$5010, Cdn$1431 and Cdn$421 respectively).[4] It also showed that the economic burden attributable to insomnia-related work absences and reduced productivity is much higher than that for the treatment of insomnia.

Apart from mental and socio-economic adversity, increasing attention has been recently focused on the medical consequences of insomnia. Two studies found that

insomnia was correlated with a higher risk for hypertension,[5, 6] while 2 other studies from one cohort did not find any association of insomnia with hypertension.[7, 8] More recently, Vgontzas et al found that insomnia was not only correlated but also had synergistic effects with short sleep duration (≤5 hours) on increased risk of hypertension.[5] The same group also found that chronic insomnia with short sleep duration (≤ 5 hours) was correlated with type 2 diabetes with an odds ratio (OR) [95% CI] of 2.95 [1.2-7.0]. Using 24-hour ambulatory blood pressure (BP) measure, Lanfranchi et al found that insomniacs had decrease in nocturnal BP dipping than good sleepers.[9] Overall speaking, these results indicated that insomnia likely led to negative effects on cardiovascular and metabolic systems. However, these studies had several limitations. First, the association in most studies was based on cross-sectional design and the cause-effect relationship between insomnia and medical diseases needs further investigation. Second, as insomnia symptom is commonly found in psychiatric disorders such as depression.[10] These studies did not exclude or control for depression, which might be a confounder or mediator in the relationship between insomnia and other medical diseases as depression has been found to be an independent risk factor for diabetes[11] and cardiovascular diseases.[12] Third, none of these studies investigated its underlying mechanism in the relationship between insomnia and physical diseases. As insomnia is considered as a state of hyperarousal during not only nighttime but

also daytime (see below), [13-16] further studies on the biological correlates of insomnia are needed.

1.2 Definition of insomnia in epidemiologic study

In modern medicine, insomnia is more clearly defined with operational criteria with duration and frequency requirements. Insomnia is defined as the complaints of difficulty initiating sleep (DIS), difficulty maintaining sleep (DMS), and/or early morning awakening (EMA).[17] A duration with at least 4 weeks and daytime functional impairments are often required when diagnosing insomnia. [3, 4, 17] As proposed by International Classification of Diseases, 10th Revision (ICD-10), a frequency of ≥ 3 times/week of any subtype of insomnia symptom is required to diagnose insomnia.[18] In addition to DIS, DMS and EMA, non-restorative sleep (NRS) is also suggested by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and International Classification of Sleep Disorders (ICSD) as a subtype of insomnia.[17, 19] Nevertheless, NRS may be a non-specific complaint which could be a symptom of other sleep disorders, such as OSA, RLS, and insufficient sleep as well as other medical and psychiatric disorders.[20] Currently, NRS is considered as a complex symptom without any standard measure in its identification.[21] Most of previous

studies used various terms, such as "unrefreshing sleep", "feeling unrested upon awaking" or "light/poor sleep" to evaluate NRS. Nonetheless, Roth et al has recently suggested that NRS may be a distinct component of insomnia as compared with other three subtypes of insomnia (DIS, DMS and EMA).[22]

1.3 Epidemiology of insomnia in children and adolescents: prevalence, incidence, remission and their correlated factors

Insomnia is commonly found in general population with a prevalence of around 10%-20% in adults and 4-8% in children by restrictive diagnostic criteria (3 times/week or often).[20, 23-27] Most of the epidemiologic studies on insomnia in children were of cross-sectional design. These cross-sectional studies found that several risk factors were consistently correlated with childhood insomnia, such as age, lower socio-economic status, poor sleep hygiene, stressful life events as well as uncomfortable sleep environment. Although gender difference for adult insomnia has been well documented,[20] our previous study found that there were no differences in insomnia across different age and gender in pre-pubertal children.[27] Nonetheless, female predominance for insomnia was suggested to begin in late adolescences after puberty.[28]

The longitudinal studies on the course of insomnia (both children and adults) are presented in table 1. In recent five years, several prospective studies have focused on the long-term course of insomnia in children. All these studies were conducted in western countries which, in general, suggested that insomnia had a persistent course for a proportion of individuals. The study by Roberts et al found that the persistence of insomnia in adolescence after 12 months varied from 23.6% to 45.9% according to various diagnostic criteria of insomnia while one year incidence was 13.9% for insomnia symptoms and 5.5% for insomnia symptoms plus daytime fatigue and/or sleepiness.[29] This study also found that several risk factors, including female gender, worse perceived health and psychological dysfunction, were strongly correlated with insomnia at baseline but were much weaker or even had no statistical significance in predicting the incidence and persistence of insomnia. Another study conducted on children found that over 60% of children with DIS still had DIS complaint while 40% of children with DMS still reported DMS complaints after one-year period in a 3-wave designed longitudinal cohort.[24] Using parent-reported sleep onset latency (SOL) of at least 15 min to define DIS, Simard et al found reverse association in DIS between age at 5 months and age at 5 years old (OR (95%CI)=0.50(0.25-0.97)) but a very weak association between age at 5 months and age at 6 years old (OR

(95%CI)=1.48(1.05-2.09)).[30] In other words, the sleep onset difficulty in early childhood was rather unstable. These various studies shed some light on the characteristics of the longitudinal course of childhood insomnia. First, the usage of stricter criteria would lead to lower persistence and incidence rate of insomnia.[29] Second, persistence of insomnia seems to be age-dependent. Persistence of insomnia could not be found between infants and young children of 5 years old years but became significant in older children and adolescents. Nevertheless, more studies are needed to investigate the longitudinal course of insomnia in children and adolescents.

1.4 Epidemiology of insomnia in adults: prevalence, incidence, remission and their correlated factors

More studies have been reported on the natural history of insomniac symptoms in adults when compared with those of children. A few longitudinal studies suggested that insomniac symptoms were prevalent with persistent rates varying from 40% to 69%[31-37] and incidence rates varying from 7.4% to 23%[32-34, 36-38] according to different populations and periods of follow-up in general population. Several risk factors were found to correlate with persistence of insomnia, such as insomnia severity,[35] older age,[32] depressive symptom and physical disorders,[34, 36] physical inactivity and pain.[34]

Incidence of insomnia has been found to correlate with similar factors contributing to its persistence.[32, 34, 37, 38]

In summary, previous studies in both children and adults have found that insomnia is a common sleep problem with a considerable degree of persistence, remission and relapse rates. However, several aspects are still unclear about the longitudinal history of insomnia. First, all but one longitudinal study were conducted in western countries. Little is known about the long-term course of insomnia in non-western population. Second, although puberty was considered to have strong effects on gender differences in sleep/wake patterns and sleep problems in cross-sectional study,[28, 39, 40] little is known about the changes of insomnia symptoms of children when they are entering into their pubertal phase. Finally, only 2 adult studies had longitudinal periods over 5 years. In these regards, longitudinal course of insomnia definitely needs further investigation across different ethnic populations.

Table 1 Longitudinal studies on course of insomnia in children and adults

Authors and years	N, year(s) of follow-up	Methods	Major findings	Limitations
Children or adolescents				
Fricke-Oerkerman et al, 2007[24]	832 (9.4,10.7 and 11.7 years old), 3 waves annually follow-up	Children and parents reported questionnaires	58.6-66% persistence rate for "sometimes"/"often" sleep onset problem, 42.6-63.6% persistence rate for "sometimes"/"often" sleep maintaining problem	Lower response rate at follow-up (53% and 32% for baseline and first follow-up)
Roberts, et al; 2008[29]	3134 (11-17 years old), 1 year follow-up	Using diagnostic interviews and questionnaires based on DSM-IV insomnia criteria	Rates of persistence were 45.8% for insomnia symptoms, 34.7% with daytime fatigue or sleepiness, and 22.8% for insomnia cases. Psychological dysfunction but not socio-demographics predicted incident and persistence of insomnia	
Adults				
Breslau N et al. 1996[31]	979 (20-30 years old adults). 3.5 years follow-up	NIMH Diagnostic Interview Schedule	Incidence and persistence rate of insomnia were 13.1% and 45% respectively.	Modest sample size
Janson C, et al. 2001[34]	2602 men (age 30-69 years); 10-year follow-up	Self-designed questionnaire	Persistence of insomnia was 44%. Being overweight (OR=1.35), physical inactivity (OR=1.42), alcohol dependence (OR=1.75), psychiatric disorders (OR=8.27) and joint/low back pain were correlated with persistence of insomnia	Absence of female data
Morphy H, et al; 2007[32]	2662 (aged over 18 years); 12-month follow-up	Self-designed questionnaire	Incidence of insomnia was 15% and was correlated with baseline anxiety, depression, and pain. Persistence of insomnia was 69% and was associated with older age.	Low response rate (54.4% at baseline)

Jansson-Frojmark M & Linton SJ. 2008[33]	1746 (20 to 60 years old); one year follow-up	Self-designed questionnaire	Persistence of insomnia was 44%. The cumulative incidence of insomnia was 2.8%.	
Buysse DJ et al. 2008[37]	591 (young adults); 6 interviews for 10 years	Structured Interview	40% of subjects, insomnia developed into more chronic forms over	Modest sample size
Morin CM, et al. 2009[35]	388 (insomniacs aged 44.8 [13.9] years). Three annually follow-up	PSQI and ISI	High remission rate (54%) and relapse rate (27%) for insomnia. Persistence of insomnia was correlated with its severity, female and increasing ages.	Relative small sample size
Kim JM et al. 2009[36]	909 (> 65 years of age); 2 years follow-up	Insomnia was defined as difficulty in initiation or maintenance of sleep ≥3 nights per week over the last month.	Persistence and incidence rate were 40% and 27% respectively. Baseline depression was significantly associated with incidence but not persistence of insomnia.	Modest sample size
LeBlanc M et al. 2009[38]	464 good sleepers; 6 months and 1 year follow-up	PSQI and ISI	One-year incidence rate were 30.7% for insomnia symptoms and 7.4% for insomnia syndrome.	Relatively small sample size

1.5 Local studies on epidemiology of insomnia

Similar with findings from other countries, insomnia is also a common sleep disorder in Hong Kong Chinese. Using frequency of at least 3 times/week, our previous studies found that insomnia was correlated with increased age with 12-month prevalence of 4.0%, 11.9% and 38.2% in children, middle-age adults and elderly respectively.[23, 27, 41] Our study also found that several risk factors were correlated with insomnia, such as aging, gender (for adults but not children), lower socio-economic status and chronic medical conditions.[23, 27, 41] Using similar frequency criteria to define insomnia, Chung et al found that the prevalence of insomnia symptoms was quite high with a rate of 19.1% in Hong Kong adolescents.[42] Although local cross-sectional studies reported a similar pattern of epidemiology of insomnia with other populations, there is a dearth of longitudinal outcomes of insomnia in Hong Kong Chinese population. Our study with a small sample size of clinical samples (n=53) found that only 32% of primary insomniacs in sleep clinic reported symptoms improvement while nine out of them (17%) developed psychiatric disorders after six years' follow-up.[43] This clinical study suggested that insomnia tended to persist and predispose to future psychiatric disorders in insomniacs subjects. However, the prognosis and incidence of insomnia in Hong Kong community population is unclear.

1.6 Worldwide family study on insomnia

Family study can provide information for the association among genetic, environmental factors and the disease, and hence, plays a critical pivotal role in genetic epidemiology.[44] Furthermore, identification of familial risk of the diseases could inform more specific family targets and provide the key component for treatment and prevention programs, that may target at the family as a whole.[45] To our knowledge, there were only limited number of studies about the family history of insomniacs, and majority of them were conducted on adults in western countries (table 2).[46-52] Although these studies suggested a familial aggregation phenomenon in insomnia, however, they were limited by relatively small sample sizes, lack of adjustment for potential confounding factors or lack of a control comparison group. [15-17] Collectively, the familial aggregation of insomnia as reported by previous studies had several characteristics. First, early onset insomnia seemed to have stronger familial aggregation.[47, 48] Second, primary insomniacs had more positive family history of insomnia than insomniacs with psychiatric disorders and normal sleepers.[50] Third, our study found that there was a dose–response effect of parental insomnia on the rate of insomnia of their children with a slight predilection of maternal influences.[27] Finally, twin study showed that heritability accounted for as high as

42-57% of the variance in insomnia.[51, 52] However, this data was limited by several aspects. First, none of these family studies employed clinical interview to confirm insomnia diagnosis for family members. The accuracy of self-reported family history was found to vary across different disorders. A recent statement reported by National Institute of Health (NIH) of United States found that family history had high specificity (90%-95%) but various sensitivities (6%-95%), especially for mental illness (sensitivity ranging from 6% to 82%).[53] Family history would be overestimated with increasing frequency of the disease, increasing number of relatives, and for diseases with earlier age at onset.[54] Hence, the usage of face-to-face interview with case-control design in confirming clinical diagnosis for insomnia is of value in family study. Second, only our previous study has controlled for potential confounding factors underlying the familial aggregation in terms of socio-economic status, medical conditions and proband's behavioral problems.[27] Finally, only two studies (including ours) employed control subjects to compare the family members' recurrent rates in insomnia.[27, 49] Hence, further studies with case-control design and detailed clinical assessments for the relatives are needed to confirm the familial clustering of insomnia. Furthermore, the blindness of the clinician in interviewing the relatives would be of high value in limiting the potential ascertainment bias.

Table 2 Worldwide family studies of insomnia

Authors and years	N	Method	Major findings	Limitations
Hauri et al. 1980[47]	59 insomniacs	PSG and Actiwatch confirmed	Childhood-onset insomnia was severer and had stronger family history of insomnia (55% v.s. 39%)	Self-reported family history, small sample size; absence of control group
McCarren et al 1994[52]	2825 male twins	Qx for each participants	Heritability accounted for 42% variance of trouble staying asleep	Insomnia was not confirmed by clinical interview
Bastien et al . 2000[48]	285 insomniacs	Qx for each participants	Family history were more prevalent in insomnia onset before 40	Self-reported family history; small sample size; without control group
Dauvilliers, et al;2005[50]	181 chronic insomniacs	Detailed evaluation by clinical interview	72.7% of primary insomniacs, 43.3% of psychiatric insomniacs and 24.1% of non-insomniacs reported family history	Self-reported family history small sample size; absence of control group
Watson et al; 2006[51]	1042 monozygotic and 828 dizygotic twins	Qx for each participants	Heritability accounted for 57% variance of insomnia	Insomnia was not confirmed by clinical interview
Bonneau, et al; 2007[49]	2001 population-based sample	Qx for probands	Insomniacs report a family history of insomnia (39.1% v.s.29.0%);	Self-reported family history, psychiatric disorders were not controlled for
Zhang et al; 2009[27]	5695 children and their parents	Qx for each participants	Robust familial aggregation of insomnia with dosing effects Stronger mother-child association	Insomnia was not confirmed by clinical interview

Qx= questionnaire

1.7 Our preliminary findings of familial aggregation of childhood insomnia

In 2003, we conducted a large scale community-based epidemiologic study of sleep disorders in Hong Kong children and their parents.[27] Questionnaires were distributed to children aged 6-13 years old (grade 1 to grade 6) from 13 randomly selected primary schools in both Shatin and Tai Po district from October to December 2003.[27, 55, 56] In this study, the rates of frequent insomnia symptom (DIS, DMS or EMA ≥ 3times/week) in the past 12-month were 4.0%, 12.8% and 9.7% for children, mothers and fathers respectively. A robust familial aggregation of insomnia was found even after adjustment of the shared environmental and socio-demographic factors.[27] There was a significant dose-response relationship among the children across their parental status from neither, fathers, mothers to both parents with insomnia (3.0%, 7.1%, 9.5% and 11.9%; with ORs (95%CIs) = 2.48 (1.82-4.37), 3.42 (2.55-4.59) and 4.42 (2.42-8.10) respectively). Nonetheless, our preliminary study did not have further detailed assessment by structured interview and clinical examination for either probands or their parents. Despite our effort in controlling socio-economic status and environmental factors, some other important confounding factors were not measured. In particular, stressful life events, personality styles and psychiatric disorders, which significantly contribute to insomnia, might be shared by parents and their children in

the same family. Thus, the mechanism underlying familial aggregation phenomenon of insomnia needs further detailed clinical-biophysiological delineation.

1.8 Potential mechanisms underlying familial aggregation of insomnia

As discussed above, robust familial aggregation phenomenon has been found in insomnia across various populations. However, the investigation on the underpinning mechanism of the familial aggregation of insomnia is limited. There might be several factors contributing to the phenomenon that insomnia tends to run in family.

1.8.1 Genetic factors

The familial aggregation of insomnia suggested that insomnia might be a genetically transmitted disease, albeit familial aggregation is not equivalent to genetics as it might also be contributed by shared environmental factors. High heritability of insomnia or poor sleep reported in twin study also strongly suggested the genetic basic of insomnia.[51, 52] However, there were very limited molecular genetic studies on insomnia and most of them were focused on the rare type of insomnia—fatal familial insomnia (FFI), which is a prion disease related to mutation at codon 178 (D178N) of

prion protein (PrP) gene (*PRNP*).[57] This rare mutation is unlikely to account for the high prevalence of familial aggregation of insomnia. For the common insomnia, one study of 112 primary insomniacs found that missense mutation of GABAAβ3, which could change the function of GABAA receptor, might be correlated with insomnia. However, this mutation could only be found in one patient out of 112 insomniacs, which indicated that this mutation might not account for most of the cases. Recently, one association study found that short allele of Serotonin Transporter Length Polymorphism (5-HTTLPR) was significantly associated with primary insomnia in German population but with a relative small Odds ratio (OR) of 1.34.[58] These findings supported the genetic mechanism for insomnia and provided biological evidences in linking high comorbidity between insomnia and major depressive disorder as depression was also found to be correlated with the interaction between 5-HTTLPR and life events.[59] Nonetheless, the odds ratio value is rather low and there are likely that other multiple factors (other genes and environments) will contribute to insomnia. Further studies in other populations and other genes are warranted for better delineation of genetic basis of insomnia.

In addition, the lack of genetic study on insomnia might also be accounted by a number of factors: 1) Endophenotype, as a stable and heritable marker in

pathophysiology of disease, which bridges the gap between upstream gene and the downstream symptoms, plays a critical role in the underlying genetic predisposition.[60] However, to the best of our knowledge, no specific endophenotype has been identified in insomnia. 2) Previously, insomnia was considered as a symptom secondary to other psychiatric disorder and medical conditions rather than an independent disorder in most situations. However, the fact that insomnia needs independent treatment and insomnia tends to persist even after a good control of the primary conditions suggests that insomnia should be considered as an independent and comorbid disorder. 3) Finally, many diseases (especially complex diseases) are likely the outcomes of genetic and environmental interaction. The recruitments of both environmental and genetic factors might enhance the power to detect the statistical associations among gene, environment and disease.

1.8.2 Shared environmental factors

Epidemiologic studies have consistently suggested that insomnia was correlated with several environmental factors, such as lower education level, lower family income, unemployment, poor housing condition, life-style and daytime activities, which are highly correlated with each other and shared by the family members. Our previous

family study of insomnia also found that several factors were commonly correlated with the occurrence of insomnia in all family members, such as the noisy environment, family members' snoring and children's behavioral and medical problems.[27] The familial clustering of insomnia, however, was maintained even after controlling for these environmental and socio-economic factors, which indicated that the familial aggregation of insomnia was not mainly mediated or modulated by these factors. It is noteworthy that in this study, some other potential confounding factors running in the family were not measured and controlled, such as stressful life events and life-style. Early study demonstrated that stressful life events also aggregated in the family.[61] Hence, further study should also focus on the role of familial clustering of stressful life events and other lifestyle factors on familial association of insomnia.

1.8.3 Co-occurrence of personality and psychiatric disorders with insomnia

Insomniacs tend to have higher scores in neuroticism and internalization and showed high signs of perfectionism.[62] The role of specific personality on pathogenesis of insomnia was complex. We take neuroticism as an example here. Neuroticism was suggested as a predisposing factor of insomnia. On the other hand, subjects with high neuroticism might also overreact to stressful life events and might also more likely

suffer from mood and anxiety disorders.[63] In this situation, personality might serve as both predisposing and precipitating factors for insomnia. A recent large-scale twin study suggested that the heritability for "neuroticism" was quite high with a h^2 of 43%.[64] These findings suggested that familial aggregation of insomnia might be partially accounted by the familial aggregation of underlying personality.

On the other hand, both twin and family studies on depression and anxiety disorders suggested a robust familial aggregation of these psychiatric disorders.[65, 66] The offsprings of those probands with major depressive disorder were around 4 times more likely to have depressive episode than those subjects without parental history of depression.[66] At the same time, insomnia is a common sleep disorder comorbid with depression and anxiety. Based on previous epidemiologic studies, a recent review suggested that insomnia was more likely to be a precursor or comorbidity of depression rather than the alternative direction that depression was a precursor of insomnia.[10] The familial clustering of depression and anxiety might also contribute to the familial aggregation of insomnia or vice versa. However, to our knowledge, none of the previous family study on insomnia measures personality factors and psychiatric disorders.

Our previous studies also found familial aggregation in other sleep/wake problems, such as sleep/wake patterns,[67] habitual snoring[68] and frequent nightmares (submitted data). These sleep problems were also correlated with insomnia in both children and adults. Hence, the familial aggregation of insomnia might also contributed by other sleep problems.

The mechanisms underlying the familial aggregation of insomnia are more complex than we have expected before. The clear definition of its phenotype and its comorbidity, the documentation of the potentially correlated psychobiological factors as well as the external environments are the first and most crucial step in unraveling the familiality of insomnia. On the other hand, although insomnia was more likely to persist over time, especially in adults (40-60%), its waxed and waning course may need careful elucidation of the symptoms.

1.9 Could hyperarousal serve as a potential endophenotype of insomnia?

In the past, insomnia was considered to be a disorder of sleep loss or sleep restriction. [69] Therefore, pharmacologic and psychotherapeutic managements of insomnia aimed to extend the sleep duration and sleep efficiency and these two parameters were the

major indicators for treatment effect. Recent studies on the pathophysiology of insomnia demonstrated that insomnia is not simply a disorder of sleep loss during nighttime, but is likely a condition related to the hyperarousal state of the subjects. Two recent reviews by Riemann[53] et al and Bonnet and Arand[70] have updated the evidences for hyperarousal concept in the pathogenesis of insomnia in terms of physiological, genetic and psychological findings. It is widely considered that hyperarousal may be a state of hypervigilance of emotion and/or physiology with consequent difficulty initiating and maintaining sleep at night. [53, 70 71-73] Among the multiple risk factors, some of the personality attributes, lifestyle and coping style have been suggested to predispose and perpetuate insomnia and hence were considered as psychological index for hyperarousal.[74-78]

1.9.1 Insomnia and stress-related hormones: consistence and controversy with hyperarousal hypothesis

In the past decade, increasing number of studies focused on the relationship between sleep and stress-related hormones. Hypothalamus-pituitary-adrenal axis (HPA) and locus ceruleus-norepinephrine-autonomic system are two major components involving in stress response.[79] HPA axis is considered as the most important mediator

of the organism's response to stressor.[80] Most studies employed cortisol, which has been widely used as a target hormone in research of stress for over thirty years, as an indicator to investigate the activity of HPA axis. However, reliable measure of cortisol level is a complex issue due to the circadian variability of its secretion. The diurnal cortisol level is characterized by increasing level on awakening at the end of nocturnal sleep, surging at 30-45 min after awakening (cortisol awakening response, CAR), gradual decrease during daytime, reaching its nadir during midnight and then increasing gradually in the latter half of night.[81] Although various measures were applied to assess the function of cortisol secretion, such as urinary, salivary and blood sample as well as challenge test (e.g., dexamethasone suppression test and stress-induced test), [53] no single method can probably capture the whole profile of cortisol secretion due to its variability and multiple determinants.

The relationship between insomnia and cortisol level is understandably inconsistent. As early as in 1998, Vgontzas et al found that 24-hour urinary free cortisol level was positively correlated with total wake time as measured by PSG in 15 chronic insomniacs.[16] However, the absence of control group in this study limited its generalization of the conclusions. The same group later reported that insomniacs had higher evening and nocturnal cortisol and adrenocorticotropic hormone (ACTH)

levels when compared with normal controls.[82] This finding was later replicated in severe chronic primary insomniacs (n=7) by Rodenbeck et a.[83] Recently, another study performed on 64 healthy children found that afternoon cortisol level was correlated with objective sleep quality and subjective sleep problems after controlling for demographic variables.[84] However, the correlation between insomnia and increased cortisol level could not be replicated by the other 2 studies.[85, 86] Using similar design with Vgontzas's study, [82] neither increased evening coritsol levels in insomniacs nor correlation between cortisol levels and sleep parameters in PSG was found.[86] It should be noted that all of the above mentioned studies were of relatively small sample sizes and most of them only focused on evening or nocturnal cortisol level. Other studies using more sophisticated design on cortisol sampling across several time series upon awakening to measure cortisol awakening response (CAR) suggested that the relationship between insomnia and HPA axis activity was much more complicated. Backhaus et al found that insomniacs (n=14) had decreased awakening salivary cortisol than age-sex matched normal controls (n=15) and awakening cortisol level was negatively correlated with nighttime awakenings and PSQI score in overall sample.[87] A recent report from the Whitehall II Study, [88] which recruited large sample (n=2751) of general population, showed that self-reported sleep disturbances were independently correlated with flatter diurnal slope in cortisol

level which was mainly due to increased evening cortisol rather than decreased awakening cortisol as suggested by Backhaus et al.[87] In 114 healthy middle-aged normal adults, our previous study did not found any association between 3-day average salivary cortisol level and sleep quality (sleep efficiency, SOL and WASO) as objectively observed by actigraphy (submitted).

Overall, the relationship between insomnia/poor sleep and cortisol was inconsistent. Hence, our understanding is still very limited as regarding to the relationship between insomnia/poor sleep and cortisol level. Several aspects should be observed in the future studies to disentangle the relationship between insomnia and cortisol profile. First, the relationship between insomnia and HPA axis dysfunction might be causative or consequential or bidirectional. Second, sleep loss induced by insomnia might be a potential confounding cause of changes of cortisol level. Previous studies suggested that the effects of sleep deprivation in experimental studies on cortisol level varied from mild elevation, no effect to slight decrease (see more details in review by Meerla et al [89]). However, some observational studies including our own data found that subjects with shorter sleep duration had lower cortisol at awakening. [87, 88] Early experimental study also found that infusion of ACTH or cotisol would disrupt sleep in normal subjects.[90] Exogenous CRH could increase EEG frequency in rats as well as

light sleep, awakenings and decrease sleep wave sleep in man.[91, 92] Third, most of the previous studies (except for Whitehall II study) investigating relationship between insomnia (poor sleep) and cortisol had limited sample size. Larger sample size would allow researcher to investigate the relationship between cortisol and different subtype of insomnia, such as co-morbid insomnia vs primary insomnia.

CHPATER TWO

SUBJECTS AND METHODS

2.1 Summary of study designs

This is a prospective study with 3-phase design. At baseline study, we have conducted a cross-sectional study in 2003, which aimed to study the sleep/wake patterns and sleep problems in primary school children and their parents.[27, 56, 67, 93] In brief, with the help of school teachers, a total of 9172 questionnaires were delivered to the children aged 6-13 years old (grade 1 to grade 6) from 13 randomly selected primary schools in Sha Tin and Tai Po districts in 2003. A total of 6447 children (70.3%) with 10381 parents (5404 mothers and 4977 fathers) returned their questioners.[27, 93] Among 6447 children recruited into baseline study, 5872 of them (90.4%) left their contact.

1) In phase 1, we aimed to follow up this cohort to explore the longitudinal changes of the sleep/wake patterns and sleep problems in both children and their parents. We also aimed to longitudinally explore the medical and psychiatric consequences of poor sleep in this cohort.

2) In phase 2, we aimed to confirm the familial aggregation of insomnia and its potential underlying mechanism in a case-control study by a detailed clinical interview with extensive psychometric and anthropometric measures. We also aimed to test the gene-environment interaction hypothesis on the pathogenesis of insomnia.

3) In phase 3, we further measured the objective and subjective sleep quantity and sleep quality by 3-day actigraphy and sleep questionnaire. In addition, one-day serial salivary cortisol and 24-hour urinary cortisol would be collected. The aim of this study was to explore the potential biological markers of insomnia.

2.2 Hypotheses of this study

The hypotheses in phase 1 were as follows:

1) Insomnia would be persistent in Chinese adolescents and adults as similar to those of Caucasians. Several correlated factors of insomnia which were found in cross-sectional study might also predict the persistence and/or incidence of insomnia

2) Insomnia might be a predictor for several medical and psychiatric disorders after 5 years.

The hypotheses in phase 2 were as follows:

1) Insomnia has significant familial aggregation phenomenon as found in our previous study.[27]

2) Life event, as a risk factor for insomnia, might exert particularly robust effects on the occurrence of insomnia in those subjects with positive family history.

3) Insomniacs would have higher neuroticism personality than non-insomniacs and the personality trait might also modify the severity of insomnia.

4) Insomniacs would have higher psychological and somatic distress than non-insomniacs and the severity of insomnia would be correlated with the severity of the distress.

5) Insomniacs would have poor quality of life than non-insomniacs and the severity

of insomnia would be correlated with the decreases of several domains of quality of life.

The hypotheses in phase 3 were as follows:

1) Insomniacs would have poor subjective sleep quality than non-insomniacs. The objective sleep quality as measured by actigraphy might also differ between insomniacs and non-insomniacs.

2) As a heritable disorder, insomnia would have one or more biological markers, which might serve as endophenotypes of insomnia.

2.3 SUBJECTS AND METHODS IN PHASE ONE PROSPECTIVE FOLLOW-UP STUDY

2.3.1 Subjects in phase one

This study is part of an ongoing epidemiologic study about sleep problems among Hong Kong Chinese children and their parents, which started in 2003[27, 55, 56] and

followed up in 2008-2009. The protocols of this study at both baseline and follow-up were approved by the Institutional Ethics Review Committee of The Chinese University of Hong Kong. All participants gave their consent to this study (for each phase). Figure 1 delineates the recruitment of the subjects. Among 5872 families who left their telephone numbers, a total of 3416 were contactable at the time of follow-up, while 55 of them refused to participate into this study during telephone contact and 3361 (98.4%) of them gave oral consent and their home address for sending the sleep questionnaires. The package with brief questionnaires for adolescents, fathers and mothers as well as their sibling(s) aged over 6-year old (if any) were mailed to the subjects according to the address provided. The families would be reminded on 2-4 weeks interval. If the questionnaires were not returned after 2 months, we made a phone call again to the families to confirm that they had received the questionnaires and reminded them to return the questionnaires. If the questionnaires were not returned after another 2 months, a remind letter would be sent. Finally, a total of 1611 adolescents out of 3416 contactable children (47.2%) returned their packages of questionnaires at the follow-up study. A total of 1404 mothers and 1237 fathers were recruited into both baseline and follow-up. 1055 siblings of adolescents aged above 6-year old were newly recruited into the follow-up survey. Table 3 depicts the individual tools and their corresponding purposes of measures.

Figure 1 Recruitments of subjects in phase one

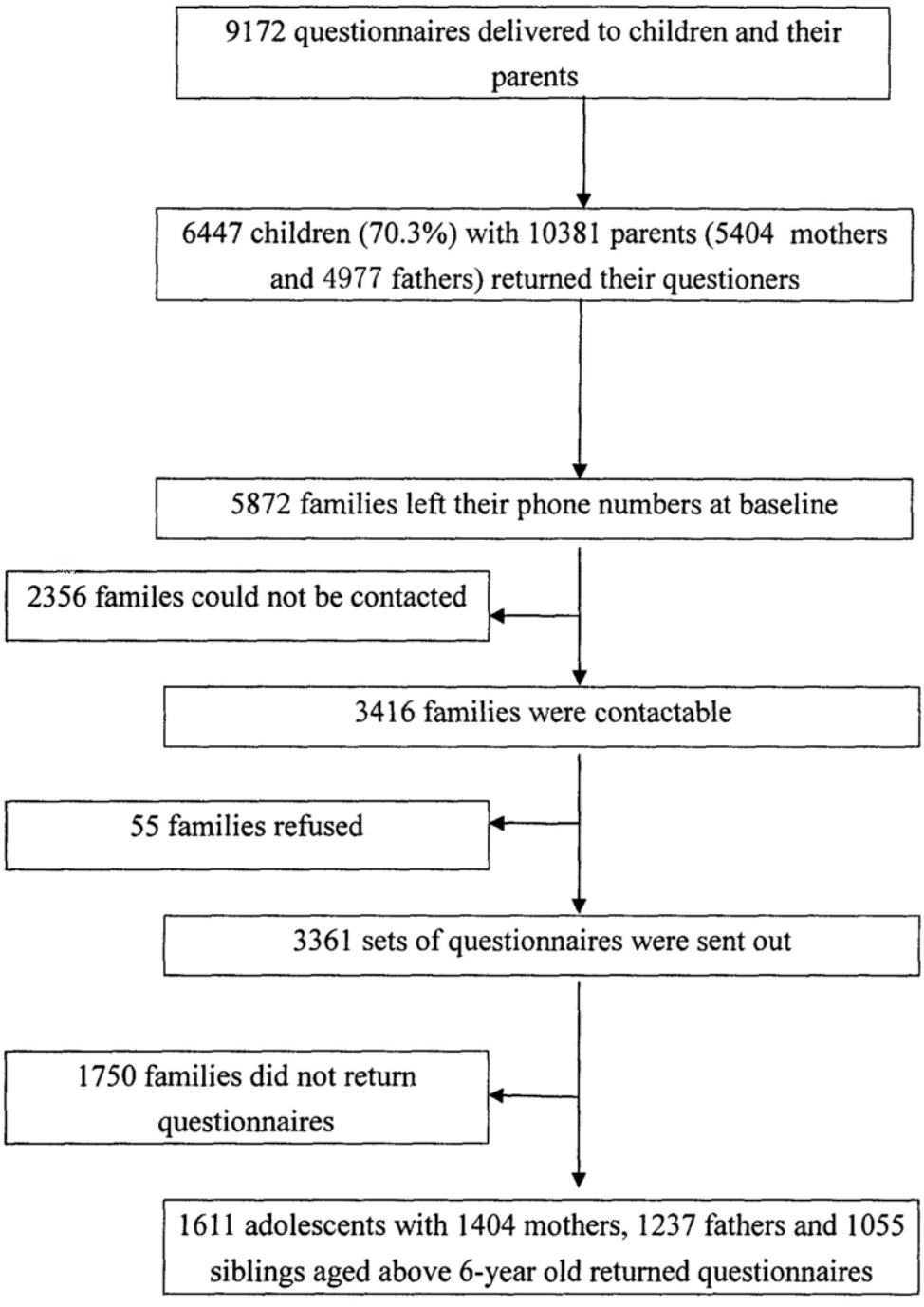


Table 3 Measures and their corresponding indications in different phases of study

Measures	Aims
	Phase 1
Sociodemography*	Socio-demography
Sleep related symptoms*	Frequency of various sleep related symptoms
Insomnia Severity Index*	Severity of insomnia symptoms
Tanner stage scale[#]	Puberty assessment
Health condition assessment*	Common and/or chronic diseases
	Phase 2
Insomnia Severity Index*	Severity of insomnia symptoms
Pittsburgh Sleep Quality Index*	Subjective sleep quality
Ford Insomnia Response to Stress Test*	Trait vulnerability for the propensity of insomnia
Beliefs and attitudes about sleep*	Subjects' beliefs and attitudes about sleep
NEO Five Factor Inventory*	Personality assessment
Chinese Adolescent Life Event Scale[#]	Life event in adolescents
Short Form Health Survey*	Health-related quality of life
Visual analogue scales for pain*	Severities of different kinds of pain
Somatic Symptom Inventory*	Somatic symptoms
Beck Depression Inventory*	Severity of depressive symptoms
HADS-Anxiety*	Severity of anxiety symptoms
Tanner stage scale[#]	Puberty assessment
DISC-IV[#]	DSM-IV adolescent disorders
SCID-I[$]	Screening of major Axis I DSC-IV diagnosis
	Phase 3
Three-day actigraphy*	Objective sleep quantity and quality
Three-day sleep questionnaire*	Subjective sleep quantity and quality
Serial salivary cortisol (7 time points) *	HPA axis activity
Twenty four-hour urinary cortisol*	24-hour urinary excretion of cortisol

* Both parents and adolescent; [#] Adolescents only; [$]Parents only

2.3.2 Measurements in phase one

2.3.2.1 Sleep questionnaire for adolescents and their sibling(s)

Our questionnaire at baseline study (2003) consisted of 54 items on demography, sleep environment, family information, sleep habits and problems.[27] The questionnaire at the follow-up study (2008) consisted of 49 items on socio-demography, sleep environment, sleep habit and problems which was similar to the original one but with slight amendments. For evaluation of more details on insomnia and pubertal status, insomnia severity index (ISI) and tanner stage scale for puberty was added into the package of questionnaire (items 37, 38 and 47). In keeping with previous study, all information of sleep problems (items 12-36) was based on the report over past 12 months.

Insomnia Severity Index (ISI) is a 7-item questionnaire assessing the subtype, severity, and impact of sleep difficulties which has adequate psychometric properties.[94, 95]

Pubertal status were assessed by tanner pubertal self-assessment questionnaire,

which is an adaptation of an interview-based puberty-rating scale,[96] and includes scores for each of five items rating physical development, an overall maturation measure, and a categorical maturation score designed to be similar to Tanner staging categories. [96, 97] This scale has excellent agreement between self-reported and peer-rated assessments in Chinese adolescents.[97]

General health and chronic diseases assessment

Subjects were asked: how is your general health condition in recent one year? The responses could be: 1) very poor; 2) poor; 3) general; 4) good; and 5) very good. The participants were also asked whether they "often" had the following six common diseases in recent one year, including allergic rhinitis, nasosinusitis, otitis media, asthma, adenoid-tonsillitis and laryngopharygngitis. For assessment of chronic medical conditions, the subjects were also asked "have you been diagnosed to have the following diseases by a doctor and/or need treatment?" These chronic diseases included hypertension, eye diseases, hypercholesterolemia, arthritis, epilepsy, cardiovascular diseases, diabetes, chronic lung diseases, psychiatric/mood disorders, renal diseases, chronic pain, eczema, gastroesophageal reflux disease and cancer.

2.3.2.2 Sleep questionnaire for parents

The questionnaire consisted of 25 items and 41 items on demography, sleep environment, sleep habit and problems at 2003 and 2008 respectively. For evaluation of more details on insomnia, daytime sleepiness and mental health status, Insomnia Severity Index (ISI), Epworth Sleepiness Scale and General Health Questionnaire-12 were added into the package of questionnaire as independent items (items 32, 33 and 39). The information of sleep problems (items 12-36) was based on the past 12 months.

2.3.3 Environmental and socio-demographic risk factors assessments

In order to control the confounding factors underlying the familial aggregation of insomnia, sleeping and family environment, socio-economic status as well as medical and neurobehavioral conditions of the family members were also analyzed. Socio-economic status assessments included several binary or ordinal questions, such as parental occupation (employed or unemployed), parental educational level (<12 years or ≥12 years), parental marital status (married and cohabited vs. divorced, widowed or single), and family income (less than or equal to 15,000/month vs. more

than HK$15,000/month; HK$7.8 = US$1). Hyperactivity, frequent temper outbursts, and chronic medical conditions of children were rated by parents as ''yes" or ''no."

2.3.4 Definition of insomnia and subthreshold insomnia in phase 1

The items on insomnia were assessed according to three basic subtypes of insomnia. DIS, DMS and EMA were rated as ''during the past 12 months, how often do you have (1) difficulty falling asleep? (2) sudden awakening during sleep and difficulty returning to sleep? (3) early morning awakening and could not fall asleep again?" All questions about insomnia were rated in a 5-point Likert scale (''0" = never, ''1" = less than once per month, ''2" = 1–3 times per month, ''3" = 1–2 times per week, ''4" = equal to or more than 3 times per week). Those subjects with any subtype of insomnia $\geq$3 times/week were defined as insomniacs while those subjects with any subtype of insomnia $\geq$1 time/week but < 3 times/week were defined as subthreshold insomniacs. As NRS is a non-specific symptom, this study did not recruit this symptom into analysis.

2.3.5 Statistical methods in phase 1

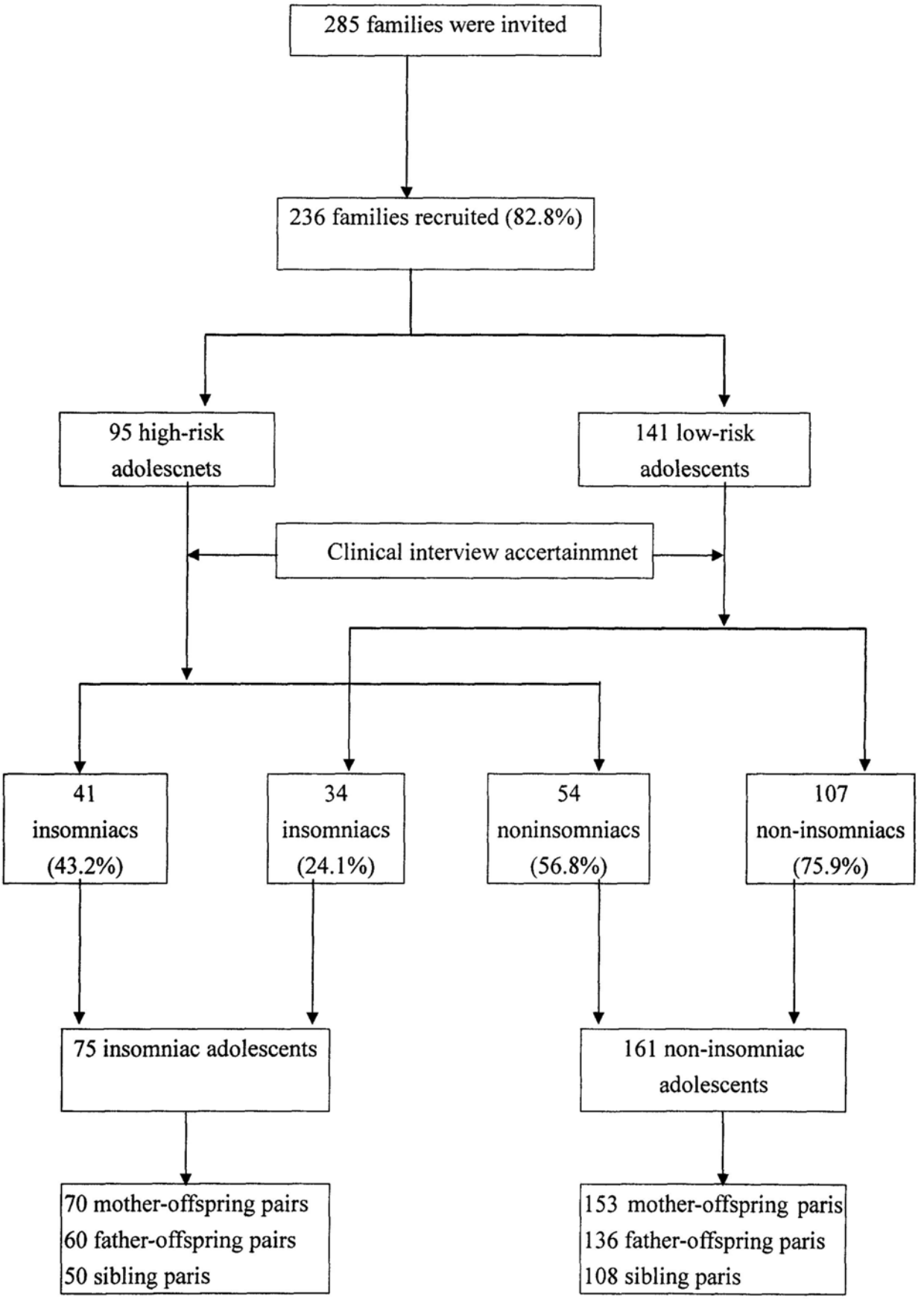
Descriptive statistics were presented as percentages for discrete variables and as means (standard deviation) for continuous variables. Chi-square statistic was used to compare the prevalence of insomnia for adolescents and their parents between baseline and follow-up. Logistic regression (forward likelihood) analysis was used to determine the associations between insomnia and risk factors after adjusting for individual group's age, family income, parental marital status and housing type, as these factors were directly shared by all subjects. The association between children's insomnia and maternal insomnia were measured by logistic regression analysis (children's insomnia as dependent variable), with forward likelihood method after adjusting for the effects of socio-demographic factors (age, sex, family income, parental occupation, parental education), family and sleeping environment (shift working status of parents, frequent snoring of family members, sharing bed, sharing room, and noisy home), medical and neurobehavioral conditions of the children (hyperactivity, frequent temper outbursts and chronic medical conditions). Same analysis was repeated for the association between children's insomnia and paternal insomnia. P-value of less than 0.05 was considered as a statistically significant level. Statistical Package for the Social Sciences (SPSS) 16.0 for windows (SPSS Inc, Chicago, IL) was used for all statistical tests.

2.4 SUBJECTS AND METHODS IN PHASE TWO STUDY

2.4.1 Subject recruitments

In phase 2 study, families were selected using a stratified random sampling procedure in which both high-risk and low-risk adolescents were identified as based on phase 1 questionnaire (figure 2). All those adolescents with DIS, DMS, and/or EMA of at least 3 times/week and/or usual sleep onset latentcy $\geq$ 30 minutes at follow-up study (phase 1) were invited into phase 2 study as high-risk probands while those subjects without any DIS, DMS and EMA ($\leq$3 times/week) at follow-up study were randomly selected from the database as low-risk controls. The parents and their siblings over 6 years old of the selected probands were also invited to participate in phase 2 study. A total of 285 probands were contacted and 236 of them finally attended with a response rate of 82.8%. Among those adolescents who were participated in phase 2 study, 95 were high-risk and 141 were low-risk (figure 2). Finally, a total of 75 adolescents were identified as insomniacs in clinical interview and the left were idenfitied as non-insomniacs.

Figure 2 Recruitments of subjects in phase tow



2.4.2 Measurements

Each subject recruited will be assessed by a structured clinical interview, a set of more detailed psychometric questionnaires and anthropometric measures. The questionnaires (table 3) for adolescents and their siblings consisted of items on demography, sleep environment, family information, sleep habits and problems, NEO-FFI, Adolescent Self-Rating Life Events Check List (ASLEC), Morningness–Eveningness Questionnaire, puberty-rating scale, sleep diary, SF-36, DBAS, PSQI, and ISI. The questionnaires for parents consisted of items on demography, sleep environment, sleep habits and problems, NEO-FFI, Life Event Scale, Morningness–Eveningness Questionnaire, sleep diary, sleep log, SF-36, DBAS, PSQI and ISI. In order to diagnose the comorbid psychiatric disorders, Structured Clinical Interview for DSM-IV Axis I Psychiatric Disorder (SCID) will also be employed for the parents and sibling(s) over 18 years old while Diagnostic Interview Schedule for studying Children-Version 4 will be employed for adolescents (probands) and their sibling(s) below 18 years old. Blood samples will be drawn from all subjects in phase 2 for basic blood and lipid profiles assessment on the day of interview. Blood pressure, height, body weight, waist circumference and bio-impedance will be measured.

2.4.2.1 Sleep quality assessment tools

2.2.2.1.1 Insomnia Severity Index (see in page 34)

2.4.2.1.2 Pittsburgh Sleep Quality Index

The PSQI is a 19-item questionnaire evaluating sleep quality and disturbances over a 1-month time interval.[98] A total score higher than 5 suggests poor sleep quality. The PSQI is of adequate Psychometric properties, with indices of sensitivity of 89.6% and specificity of 86.5% for psychophysiological insomnia.[99] Chinese version of PSQI has good validity and reliability in primary insomniacs and normal subjects.[100]

2.4.2.1.3 Ford Insomnia Response to Stress Test (FIRST)

Recently, a questionnaire known as 'Ford Insomnia Response to Stress Test (FIRST) was constructed to measure the vulnerability for the propensity of insomnia response to different stressful situations. [101] FIRST consists of 9 items which was designed to assess how easy insomnia will occur upon 9 different situations. Participants express on a four-point Likert-scale in the following manner "*not likely* = 1, *somewhat likely* =

2, *moderately likely* = 3, and *very likely* = 4." The higher score of FIRST, the more vulnerability to insomnia the subjects have. The FIRST (Chinese version) was translated into Chinese by a native Chinese speaker with bilingual fluency in both English and Chinese, which was then translated back into English by another bilingual proficient translator. As the first (before an important meeting in the next day) and sixth (after having a bad day at work) items are not applicable to adolescents, these two items in adolescents were re-phrased into "before an important examination in the next day" and "after having a bad day at study", respectively. Adequate psychometric properties were found in Chinese version of FIRST in both adolescents and middle-aged adults in this cohort (unpublished data).

2.4.2.1.4 Beliefs and attitudes about sleep

The Dysfunctional Beliefs and Attitudes About Sleep Scale (DBAS) consists of 30 statements exploring subjects' beliefs and attitudes about sleep. The DBAS has good internal consistency and discriminative ability.[102] For each item in Chinese version of DBAS, subjects express agreement or disagreement on a five-point Likert type scale ranging from "strongly disagree" (1) to "strongly agree" (5). The total score of DBAS

ranges from 30 to 150. High scores on the DBAS are indicative of high dysfunctional beliefs about sleep.[103]

2.4.2.2 Personality assessment

All subjects will be assessed by NEO-FFI (NEO Five Factor Inventory), which is available in Chinese version. The NEO-FFI consists of 60 items, 12 for each of the "Big Five" variables, including neuroticism (N), openness (O), conscientiousness (C), agreeableness (A), and extraversion (E). For each item, subjects express agreement or disagreement on a five-point Likert type scale ranging from "strongly disagree" (1) to "strongly agree" (5). High consistence in five factors model of personality was found across diverse cultures including Chinese adults.[104] The Chinese version of NEO-FFI had satisfactory psychometric properties in adolescents.[105]

2.2.2.3 Life events assessment for adolescents

Adolescent Self-Rating Life Events Check List (ASLEC) will be employed in this study to assess the life events in our study in adolescents. Chinese Adolescent Life Event Scale contains 27 items with adequate test-retest reliability and criterion-related

validity.[106]

2.4.2.4 Health-related quality of life assessment

Short Form Health Survey (SF-36)[88] was employed to measure the quality of life. SF-36 is a well-known generic instrument for measuring the health related quality of life (QoL).[1] The Chinese (Hong Kong) version of SF-36 Health Survey was found to be a reliable and valid measure of quality of life in Chinese.[107]

2.4.2.5 Pain and somatic symptoms assessments

2.4.2.5.1 Visual analogue scales for pain (VAS-pain)

100-mm visual analogue scales were employed to assess the severities of overall pain, headache, back pain, shoulder pain, time in pain while awake as well as the amount that pain interfered their daily activities.[108] The pain severity of visual analogue scales (overall pain, headache, backache as well as neck and shoulder pain) was anchored by "no pain" on one end and "as severe as I can imagine" on the other. The term "time in pain" visual analogue scale was anchored by "none" and "all the time."

2.4.2.5.2 Somatic symptoms assessment

Somatic symptoms (including both painful and non-painful items) were measured by the 28-item Somatic Symptom Inventory (SSI-28), where each item was rated according to how much it bothers the patient over the last one week on a likert type of scale from 1 (not at all) to 5 (a great deal).[109] The pain subscore (SSI-pain) was derived by calculating the average score over 7 pain-related items (including items 2, 3, 9, 14, 20, 27, and 28), and the somatic subscore (SSI-somatic) used the remaining 21 items.

2.4.2.6 Mood and anxiety symptoms assessments

2.4.2.6.1 Assessment of depressive symptoms

13-item Beck Depression Inventory (BDI) was employed to assess the depressive symptoms of participants,[110] which is a four-point scale consisting of 13 items to evaluate the severity of depressive symptoms. The total score of BDI ranges from 0 to 39.

2.4.2.6.2 Assessment of anxiety symptoms

The hospital anxiety and depression scale (HADS) was employed to assess the anxiety and depressive symptoms of the subjects. The Chinese version of HADS demonstated satisfactory psychometric properties in both adolescents[111] and adults[112].

2.4.2.7 Puberty assessment for adolescents

Tanner pubertal self-assessment questionnaire was also employed in phase 2 in view of the time gap between phase 1 and phase 2.

2.4.2.8 DSM-IV diagnostic interview for the adolescent and their parents

2.4.2.8.1 Diagnostic Interview Schedule for Children-Version 4 (DISC-IV)

All the adolescents recruited in this study underwent an interview with Diagnostic Interview Schedule for Children-Version 4 (DISC-IV) to estimate the DSM-IV psychiatric disorders for all subjects aged under 18 years. DISC-IV generates more

than 30 DSM-IV child/adolescent diagnoses based upon a 12-month time-frame, which has good reliability and validity.[113] The Chinese version of DISC-IV was found to have comparable test–retest reliability with the original English version.[114]

2.4.2.8.2 Semi-structured Clinical Interview for DSM-IV Axis I Psychiatric Disorder (SCID)

Semi-structured Clinical Interview for DSM-IV Axis I Psychiatric Disorder (SCID)[115, 116] was used for screening of major Axis I DSC-IV diagnosis for all adults subjects aged over 18 years. SCID interview was administrated by experienced psychiatrists for the presence of each disorder in the past month and lifetime occurrence.

2.4.2.9 Anthropometric measure

All participants underwent anthropometric measurements at 9 a.m upon arrival at hospital for clinical assessment after at least 8 hours fasting. Weight and height were measured with subjects wearing light clothing but not wearing shoes. Waist circumference was measured at the midpoint between lower ribs and iliac crest. Hip circumference was measured horizontally at the level of the largest lateral extension of

the hips or over the buttocks. Weight was measured to the nearest 0.1 kg (Tanita physician digital scale, Tanita Corp., Tokyo, Japan) while height was measured to the nearest millimeter for once. Body mass index (BMI) was calculated as weight divided by height square (kg/m^2). Blood pressure and pulse were measured thrice after at least 15 minutes rest period in sitting position and were averaged according to three recordings. Bioelectrical impedance method was used to estimate the body fat percentage of the subjects.

2.4.2.10 Heart rate variability (HRV)

The subjects were required to lie down on the bed in a quiet room and keep awake for 10 minutes. The heart rate samples were collected by POLAR WearLink (POLAR RS800^{TM}). The most stable consecutive 5 minutes recording was used for HRV analysis. We used Fast Fourier transformation (FFT) to perform frequency domain HRV analysis. The following frequency domain indices were calculated in this study: low frequency power (LF)- the power in the low frequency range (0.04-0.15 Hz), high frequency power (HF) – the power in the high frequency range (0.15-0.40 Hz), and the LF/HF ratio. The following time domain HRV indices were recruited: RRSTD – the standard deviation of all RR intervals (ms), RMSSD – the square root of the mean

of the sum of the squares of differences between adjacent RR intervals (ms), and Mean HR – the mean heart rate (beat per minute (BPM)). LF refers to a mixture of sympathetic and parasympathetic modulation, HF refers to the parasympathetic modulation, and LF/HF ratio represents the balance of sympathetic and parasympathetic modulation. Lower HF and higher LF/HF ratio are considered as an indicator of an autonomic balance of more sympathetic and less parasympathetic modulation. The time domain measures were used to estimate the overall variability of RR intervals. RRSTD was highly correlated with the total power of the frequency domain analysis, while RMSSD was highly correlated with the HF of the frequency domain analysis.[117]

2.4.3 Statistical methods in phase 2

Descriptive statistics were presented as percentages for discrete variables and as means (standard deviation) for continuous variables. To analyze the familial aggregation for dichotomized traits (such as insomnia diagnosis), chi-square statistic was first used to compare the rate of insomnia for the parents and siblings of those adolescents with and without insomnia. To analyze the familial aggregation for

continuous traits, Pearson correlation analysis and Spearman correlation analysis were first used to analyze the correlation in different traits between family members (father-proband pair, mother –proband pair, father-mother pairs and sibling pair) for parametric and non-parametric data respectively. Odds ratios were further estimated by Logistic Regression models and Generalized Estimating Equation (GEE) models for relatives to investigate the familial clustering of insomnia after adjusting for age, proband's gender and psychiatric disorders as well as paternal/maternal/sibling mental illness.

The comparison between insomniacs and non-insomniacs with respect to socio-demographic and clinical characteristics was performed by independent sample *t*-test, Mann-Whitney U test, and chi-square test as appropriate. To compare the independent differences for each psychometric measures (such as BDI, HADS anxiety, NEO-FFI personality) and other biological tests between insomniacs and non-insomniacs, analysis of covariance (ANCOVA) was employed to adjust for potential confounding effects of other sociodemographic and clinical variables, including categorical variables (sex, marital status, educational level and occupation) and continuous variables (age).

Multiple linear regression analysis with enter method was used to adjust for relevant covariates and to determine the independent correlates.

Except for those specified, P-value of less than 0.05 was considered as a statistically significant level and SPSS 16.0 for windows (SPSS Inc, Chicago, IL) was used for all statistical tests.

2.4.4 Estimation of heritability for traits

The additive and narrow sense heritabilities of all phenotypes were calculated by employing Sequential Oligogenic Linkage Analysis Routines (SOLAR) program after adjusting for individual age and proband's gender.[118] This method could distinguish the variances of specific phenotypes accounted by genetic (V_G) and environmental (V_E) component. The total variation of specific phenotype could be written as $V_P = V_G + V_E$. The narrow sense heritability (h^2) of total phenotypic variance is $h^2 = V_G/V_P$. The environmental component consists of common environmental factors, measurement errors and nonadditive genetic factors. Bivariate genetic analysis was also employed to explore the correlation between two phenotypic characteristics (p_P) as well as the genetic (p_G) and environmental (p_E) contributions. $p_P = p_G * \sqrt{(h_I^2 *}$

h_2^2) + p_E* $\sqrt{[(1-h_1^2) * (1-h_2^2)]}$. In this equation, h_1^2 and h_2^2 were referred to the heritability of traits 1 and 2 respectively. Age and gender were recruited into the model as covariates. Parameter estimation was performed by restricted maximum likelihood methods.

2.5 Methods and subjects in phase 3

2.5.1 Objective assessment for sleep quality and quantity

Sleep-wake estimation was recorded by wrist actigraphy (Mini Mitter, Actigraphy 16, OR, USA,), which was further analyzed with the Action-W software and the time-above-threshold algorithm. A threshold of 20 was used to distinguish sleep from waking as based on previous validation study. [119] Sleep parameters in actigraphy were estimated as follows:

-Time in bed (TIB): Interval between bedtime and rising up time as derived from event-marker buttons (defined as sleep quantity in this study).

- Actual Sleep duration (ASD): Interval between sleep start and sleep end, minus wakening duration.

-Sleep onset latency (SOL): Interval between bedtime and sleep start time

-Wake after sleep onset (WASO): waking duration during sleep start time and rising up time

-Sleep efficiency (SE): Actual sleep duration divided by TIB times 100% (defined as sleep quality in this study)

Subjects with SE less than 85% were considered to have poor sleep quality (poor sleepers) as defined similarly by previous study. [5]

2.5.2 Subjective sleep assessment

A questionnaire with regard to 3-day self-reported sleep quality and quantity was recorded together with actigraphy by the participants. This questionnaire includes daytime napping, subjective sleep onset latency, bedtime, wakeup time, subjective hours spent in sleep, number of awakening and average time spending on returning sleep after awakening. Subjective time in bed was calculated as interval between bedtime and wake up time. Subjective sleep efficiency was calculated as (hours spent in sleep/subjective time in bed)*100%. WASO was calculated as (time in bed – sleep time – sleep onset latency).

The sleep parameters as extracted from 3-day actigraphy and sleep questionnaire were averaged to final analysis.

2.5.3 Serial salivary cortisol

2.5.3.1 Sampling methods

The subjects recruited for actigraphy assessment will collect salivary specimen starting from the morning of day 2 or day 3 for salivary cortisol analysis. Subjects were instructed to collect salivary samples using salivettes by the participants at waking 0 min (T1), 30 min (T2), 60 min (T3) and 90 min (T4) , noon time (T5), 4:00 pm (T6) and 10:00 pm (T7).[120] The salivary cortisol analysis is used to explore the familial aggregation of cortisol activity and its role in the sleep quality and sleep duration as assessed by 3-day actigraphy and questionnaire. [120] Cortisol values outside of 3 SD from the mean in each time point were considered as outlier and hence were coded as missing data.[121] Those samples of the first 4 time points (T1-T4) collected outside a margin of 5 minutes before or after the time protocol were also considered as missing. Finally, a total of 185 and 130 salivary samples were coded as missing in adolescents and adults, which conveyed rates of 10.4% and 7.6% of total salivary

samples respectively.

2.5.3.2 Missing value estimation for salivary cortisol

The missing data in those subjects with at least two valid cortisol values were estimated by expectation maximization method in Lisrel 8.7 for Windows (Lincolnwood, IL). As age and gender might be correlated with cortisol level in initial analysis, age and gender were incorporated into the model to estimate the missing values of cortisol. In view of heterogeneity in age across different groups, adolescents and adults were estimated respectively. Table 4 shows the details of missing values of cortisol across different groups.

Table 4 Number of missing values in cortisol across different groups

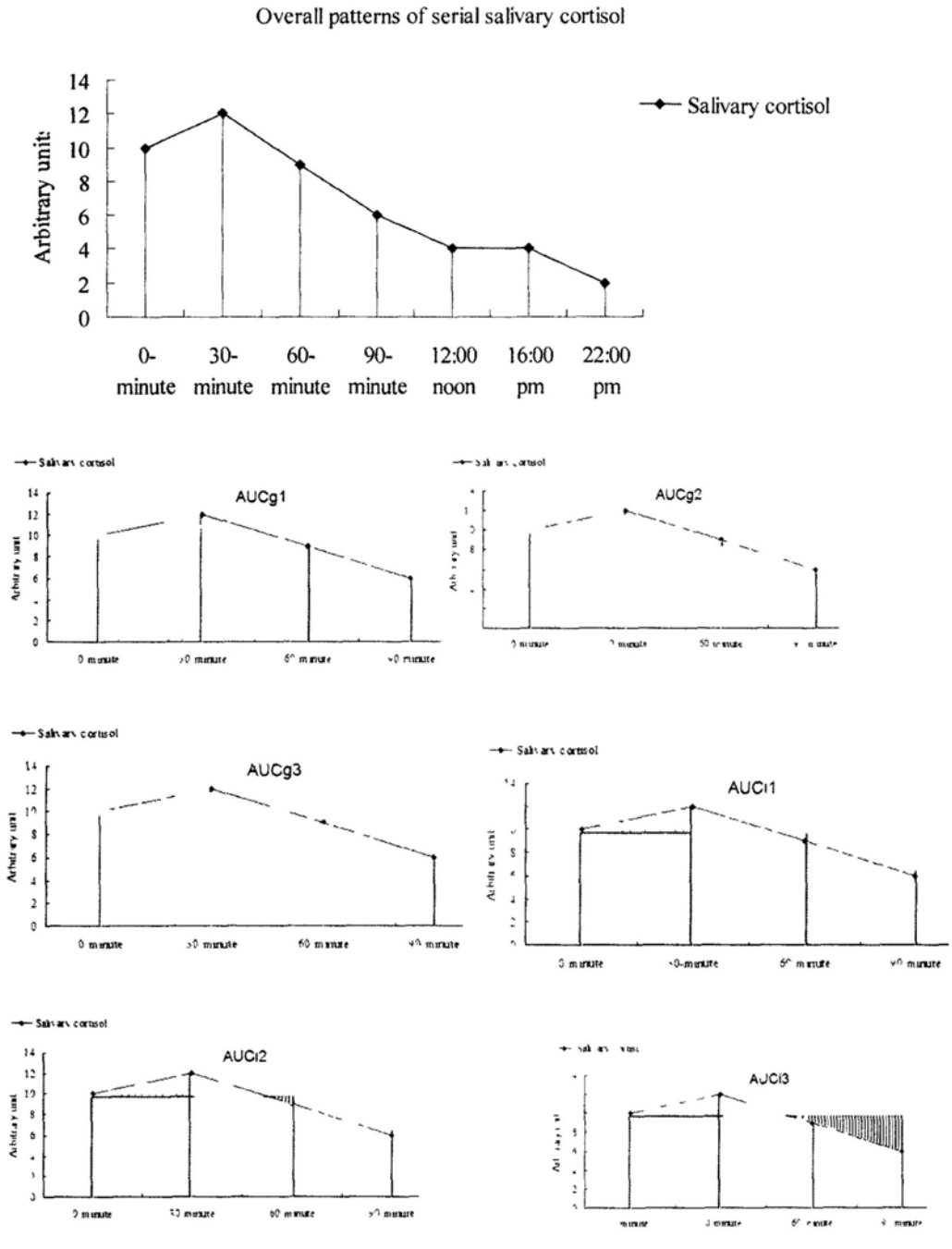
	Total	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Adolescents (n=255)	185 (10.4%)	9	31	50	46	22	11	16
Adolescents outlier nmol/L		25.3	32.0	23.1	22.3	12.6	12.3	6.2
Parents (n=244)	130 (7.6%)	6	31	30	29	14	10	10
Parents outlier nmol/L		29.2	29.8	23.3	18.2	18.8	12.6	7.4

Missing rate = Total missing number /number of subject/7

2.5.3.3 Estimation of Cortisol Awakening Response (CAR)

Two formulas proposed by Pruessner et al[122] were employed to calculate the area under the curve (AUC) of cortisol awakening response (CAR) (referring to the first four time points (T1 – T4)), one is with respect to ground (AUCg) and the other is with respect to the increase (AUCi). The AUCg was considered to estimate the total cortisol secretion (overall intensity) throughout the measuring period while AUCi was suggested to be a measure of dynamic (changes) of the cortisol awakening response.[123] As the time interval between two consecutive sample collections upon CAR was equal to 30 min, the AUCg was calculated as the sum of the cortisol levels while AUCi was calculated as AUCg – (n-1)* cortisol level at T1. AUCg1, 2 and 3 were the AUCg between T1 and T2, between T1 and T3, and between T1 and T4 respectively while AUCi1, 2 and 3 were the AUCi between T1 and T2, between T1 and T3, and between T1 and T4 respectively.

Figure 3 Serial salivary cortisol patterns and estimations of area under the curve (AUC) of cortisol awakening response (CAR)



2.5.3.4 Estimation of cortisol diurnal slope

To investigate the diurnal changes of corisol level, three time points' cortisol levels at latter part of the day (T5 – T7) were used to compare with T1 cortisol level. Diurnal slopes 1, 2 and 3 were calculated as (T1 – T5)/ T1, (T1 – T6)/ T1, (T1 – T7)/ T1 respectively.

2.5.4 Twenty four-hour urinary cortisol assessment

All subjects were instructed to collect 24-hour urinary samples during the period of actigraphic assessment so as to allow the real time association between sleep parameters and 24-hour urinary cortisol levels. All urine samples were collected into 24-hour urine bottles with 0.1L of 0.5M hydrochloric acid as preservative and stored at -20℃ before analysis.

2.3.5 Statistical methods in phase 3

Descriptive statistics were presented as percentages for discrete variables and as means (standard deviation) for continuous variables. To analyze the familial

aggregation, Pearson correlation analysis and Spearman correlation analysis were first used to analyze the correlation in different traits between family members (father-proband pair, mother –proband pair, father-mother pairs and sibling pair) for parametric and non-parametric data respectively. Genetic analysis was further employed to calculate the heritability of different traits after controlling for age and gender. Adults with TIB by actigraphy < 400 minutes were defined as short sleepers and adolescents with TIB by actigraphy < 480 minutes were defined as short sleepers (accounting for around bottom 25% of the subjects) while their counterparts were defined as normal/long sleepers. Those subjects with sleep efficiency (SE) < 85% were defined as poor sleepers while those subjects with SOL > 30 min were defined as abnormal. ANCOVA was employed to analyze the differences in serial cortisol profiles and 24-hour urinary cortisol levels between insomniacs and non-insomniacs, between short sleepers and normal/long sleepers, between poor sleepers and good sleepers and between subjects with SOL>30 min and subjects with SOL $\leq$ 30min after adjusting for age and gender. Parametric test (t test) and non-parametric test (Mann-Whitney U test) were employed to analyze the differences in sleep parameters between insomniacs and non-insomniacs. In view of the heterogeneity in age, adolescents and parents were separately analyzed.

CHAPTER THREE------RESULTS

3.1 RESULTS FOR PHASE ONE STUDY

3.1.1 Sample characteristics of both adolescents and their parents

Table 5 delineates the sample characteristics of the subjects at baseline and follow-up. Those families who dropped out from wave 2 follow-up study had slightly lower socio-economic status (lower paternal and maternal educational levels and family income, $p<0.05$) and slightly younger age in their children (9.0±1.8 vs 9.2±1.8 years, $p<0.05$ respectively) but there were no differences in any subtype of insomnia and overall insomnia prevalence between wave 1 sample and wave 1 cohort with respect to children, fathers and mothers groups (table 5). The mean age of 1611 available adolescents was 9.0±1.8 at baseline and 13.7±1.8 at follow-up respectively with an average of follow-up duration of 4.7 years. The prevalence of insomnia was higher at follow-up than baseline (6.6% vs 4.2%, $p=0.001$) in adolescent group while the prevalence of insomnia was lower at follow-up than baseline in father group (8.1% vs 10.7%, $p=0.02$) and there was a similar rate of insomnia in mother group at both baseline and follow-up (13.9% vs,11.6% $p>0.05$).

There was a higher rate of positive family history (any family member with insomnia) with insomnia (34.6%) in insomniac adolescents when compared with those without insomnia (19.9%) with an OR(95%CI) of 2.13(1.29-3.51) (p=0.004). The first degree relatives' recurrence risk for current insomnia was 14.2% for adolescents with insomnia and 8.6% for adolescents without insomnia with an OR(95%CI) of 1.75 (1.24-2.48) (p=0.002). These results suggested that there was familial aggregation of insomnia in phase 1 study.

Table 5 Sample characteristics of subjects at phase 1 follow-up study

	W1 sample	W1 cohort	W2 cohort
Children's age (years)*	9.2±1.8	9.0±1.8	13.7±1.8
Gender, male %	50.6%	49.1%	49.1%
Insomnia rate, %	4.0	4.2	6.6
Father occupation (employed),%	96.2	97.5	97.3
Father educational level, tertiary or above, %*	15.8	19.3	19.3
Mother occupation (employed) , %	41.4	40.8	50.2
Maternal educational level, tertiary or above, %*	10.0	12.7	12.7
Housing type (public), %*	59.9	54.2	53.9
Family income > HK$15,000/month, %	46.9	56.4	67.5
Parent's marital status (married or cohabited), %	92.5	93.3	91.2
Father's age (years)	43.1±5.6	43.0±5.1	47.9±5.0
Mother's age (years)*	38.8±4.7	39.3±4.4	44.3±4.4
Father insomnia, %	9.7	10.7	8.1
Mother insomnia, %	12.8	13.9	11.6

*P<0.05 between sample 1 and cohort 1 subjects.

3.1.2 Course of insomnia over 5-year interval in children

Tables 6 and 7 depict the course of insomnia in both children and their parents. The prevalence of insomnia with a frequency of at least 3 times/week was 4.2%, 10.7% and 13.9% at W1 cohort and 6.6%, 8.1% and 11.6% at follow-up for adolescents, fathers and mothers respectively. Similar incidence rate of insomnia was found across adolescents, fathers and mothers (6.2%, 5.4% and 6.8% respectively, $p>0.05$), while highest persistence rate of insomnia was found in mothers (43.8%), followed by fathers (26.9%) and adolescents (14.9%) (mothers vs adolescents OR (95%CI) = 4.43 (2.22-8.86); mothers vs fathers OR(95%CI)=2.11(1.31-3.42); fathers vs adolescents OR(95%CI)=2.1(0.98-4.52)). The persistence rates of insomnia would increase to 27.4%, 44.8% and 53.9% for children, fathers and mothers respectively when a loose criterion of insomnia ($\geq$ 1 times/week) was used.

The prevalence of DMS and EMA in adolescents group were similar between baseline and follow-up ($p>0.05$) while prevalence of DIS increased from 2.6% to 5.6% with an OR (95%CI) of (2.21(1.53-3.18)), which indicated that the increased prevalence of insomnia from children to adolescents was predominantly due to increased rate of DIS rather than DMS and EMA. The persistence and incidence rates of overall insomnia

were 14.9% and 6.2% respectively. Higher persistence and incidence rates were also found in DIS than DMS and EMA in adolescents ($p<0.05$). Among those children with DIS $\geq$ 3 times/week at baseline, 19.0% of them had DIS at follow-up while only 5.3% of those children with DMS $\geq$ 3 times/week persisted at follow-up. Interestingly, all subjects with EMA $\geq$ 3times/week at baseline remitted.

Table 6 Course of insomnia symptoms between baseline and follow-up

	Prevalence rates			W1, W2 cohort change		
Children	W1 sample	W1 cohort	W2 cohort	Incidence	Persistence	Remission
	N=6447	N=1611	N=1611			
DIS (%)	2.5	2.6	5.6	5.2	19.0	81.0
DMS (%)	1.3	1.2	0.8	0.7	5.3	94.7
EMA (%)	1.2	0.9	1.4	1.4	0	100
Overall insomnia (%)	4.0	4.2	6.6	6.2	14.9	85.1
Fathers	N=4289	N=1237	N=1237			
DIS (%)	4.0	3.7	3.9	2.4	35.7	64.3
DMS (%)	4.5	5.4	1.8	1.4	9.4	90.6
EMA (%)	4.9	5.3	4.0	3.3	19.4	80.6
Overall insomnia (%)	9.7	10.7	8.1	5.4	26.9	73.1
Mothers	N=4939	N=1404	N=1404			
DIS (%)	6.1	6.6	7.4	4.8	48.9	51.1
DMS (%)	6.6	7.7	4.0	2.8	18.7	81.3
EMA (%)	6.3	6.0	5.9	4.0	32.1	67.9
Overall insomnia (%)	12.8	13.9	11.6	6.8	43.8	56.2
Subthreshold insomnia + insomnia (≥ 1 time/week)						
Children (%)	11.1	12.9	19.2	18.0	27.4	72.6
Fathers (%)	20.5	22.7	20.2	12.8	44.8	55.2
Mothers (%)	25.4	27.6	26.2	16.0	53.9	46.1

Table 7 Course of insomnia over 5-year interval in children, fathers and mothers

	Baseline		5-year follow-up		Course over time	
		N (%)		N (%)	% (95%CI)	
Children insomnia	No	1544(95.8)	No	1448(89.9)		
N=1611			Yes	96(6.0)	6.2 (5.0-7.4)	←Incidence
Kappa=0.07	Yes	67(4.2)	No	57(0.6)	85.1(77.6-93.6)	←Remission
OR 95%CI= 2.64(1.31-5.35)			Yes	10(3.5)	14.9 (6.4-23.4)	←Persistence
Father insomnia	No	1107(89.5)	No	1047(84.6)		
N=1237			Yes	60(4.9)	5.4(4.1-6.7)	←Incidence
Kappa=0.19	Yes	130(10.5)	No	95(7.7)	73.1 (65.5-80.7)	←Remission
OR 95%CI= 4.69(2.89-7.62)			Yes	35(2.8)	26.9 (19.3-34.5)	←Persistence
Mother insomnia	No	1218(86.4)	No	1135(80.5)		
N=1404			Yes	83(5.9)	6.8 (5.4-8.2)	←Incidence
Kappa=0.21	Yes	192(13.7)	No	108(7.7)	56.2 (49.2-63.2)	←Remission
OR 95%CI= 4.19(2.89-6.07)			Yes	84(6.0)	43.8 (36.8-50.8)	←Persistence

There was no difference in incidence rate of insomnia between mothers and fathers (OR(95%CI)=1.28(0.91-1.78)) (p=0.162), while persistence rate was higher in mothers than fathers (p=0.002).

3.1.3 Factors associated with progress of insomnia in children

Table 8 depicts the adjusted relative risk (RR) for the relationship of incidence and persistence of insomnia with their associated risk factors. Several risk factors at baseline were associated with incidence of insomnia in adolescents, including lower paternal education level (AOR(95%CI) =2.49(1.13-5.51)), frequent temper outbursts ((AOR(95%CI) =1.85(1.16-2.97)) and feeling tired at daytime (AOR(95%CI) =2.17(1.24-3.77)). Although paternal smoking at baseline was correlated with incidence of insomnia of adolescents in crude analysis, it could not be recruited into the logistic model to predict insomnia after adjusting for age and gender. Chronic medical conditions (AOR(95%CI) =10.2(1.99-52.54)) and nocturnal sweating (AOR(95%CI) =5.35(1.13-25.33)) at baseline were strongly associated with persistence of insomnia in adolescents. Although familial aggregation of insomnia were found at baseline analysis, both paternal and maternal insomnia could not predict the progress of insomnia of adolescents ($p>0.05$). In view of potential confounding effects among the associated factors in relation with incidence/persistence of insomnia, all statistically significant associated factors were further tested by logistic regression model with forward likehood after controlling for age and gender. In this model, incidence of insomnia was predicted by lower paternal

education level (AOR(95%CI) =2.56(1.22-5.35)), frequent temper outbursts ((AOR(95%CI) =1.72(1.11-2.69)) and feeling tired at daytime (AOR(95%CI) =2.00(1.14-3.51)). Persistence of insomnia was predicted by chronic medical conditions (AOR(95%CI) =5.60(1.24-25.25)) but not nocturnal sweating ($p>0.05$), which indicated that the association between nocturnal sweating and persistence of insomnia was confounded by chronic medical conditions.

Table 8 Factors associated with progress of insomnia in adolescents

	Incidence		Persistence	
	Crude OR(95%CI)	Adjusted OR(95%CI)	Crude OR(95%CI)	Adjusted OR(95%CI)
Family information				
Noisy home				
Yes vs No	1.14(0.71-1.85)	---	2.00(0.34-11.6)	---
Family income (monthly)				
≤15,000 vs > 15,000 (HK$)	1.00(0.66-1.54)	---	1.61(0.67-3.89)	---
Housing type				
Public vs Non-public	1.00(0.80-1.26)	---	2.00(0.41-9.71)	---
Paternal occupation				
Employed vs Unemployed	1.13(0.27-4.80)	---	--	--
Maternal occupation				
Employed vs Unemployed	1.01(0.66-1.55)	---	1.04(0.09-10.22)	---
Paternal information				
Paternal education				
Non-tertiary level vs Tertiary level	**2.56(1.24-5.41)***	**2.49(1.13-5.51)***	0.63(0.10-4.05)	---
Paternal smoking				
Often vs No or seldom	1.66(1.01-2.72)	---	3.50(0.61-20.10)	---
Maternal information				
Maternal education				
Non-tertiary level vs Tertiary level	1.57(0.75-3.31)	---	3.50(0.58-21.08)	---
Maternal smoking				
Often vs No or seldom	1.89(0.56-6.40)	---	5.89(0.34-102.88)	---
Children's information				

Hyperactivity				
Yes vs No	1.89(1.15-3.11)	---	0.83(0.17-4.21)	---
Frequent temper outbursts				
Yes vs No	**1.81(1.17-2.80) ***	**1.85(1.16-2.97)***	3.45(0.81-14.74)	---
Chronic Medical condition				
Yes vs No	1.42(0.83-2.41)	---	**6.53(1.49-28.23)***	**10.2(1.99-52.5)***
Feeling tired during daytime				
Yes vs No	**2.18(1.25-3.79) ***	**2.17(1.24-3.77) ***	1.90(0.49-7.37)	---
Nocturnal sweating				
Yes vs No	1.32(0.76-2.27)	---	**4.71(1.05-21.04) ***	**5.35(1.13-25.33) ***
Paternal insomnia at baseline				
Yes vs No	0.80(0.36-1.78)	---	1.40(0.24-8.34)	---
Maternal insomnia at baseline				
Yes vs No	1.40(0.78-2.49)	---	0.79(0.18-3.41)	---

Adjusted OR: Adjusted for age and gender

-- Without significance in adjusted model

* $p<0.05$

3.1.4 Course of insomnia over 5-year interval in parents

Tables 6 and 7 report the course of insomnia in parents. The prevalence of overall insomnia with a frequency of $\geq$ 3 times/week was 10.7% and 13.9% at baseline and 8.1% and 11.6% at follow-up in fathers (baseline vs follow-up OR(95%CI)=1.36(1.05-1.76), P<0.05) and mothers groups (baseline vs follow-up OR(95%CI)=1.22(0.99-1.52), P=0.006) respectively. DIS had highest persistence rate (35.7% in fathers group and 48.9% in mothers group), followed by EMA (19.4% in fathers group and 32.1% in mothers group) and DMS (9.4% in fathers group and 18.7% in mothers group) respectively. Although similar prevalence of different subtypes of insomnia was found at baseline, lower incidence and persistence rate of DMS were found in both fathers and mothers groups when compared with other 2 subtypes of insomnia, which led to lower prevalence of DMS at the follow-up than baseline (OR (95%CI) =3.09(1.99-4.80) for fathers and OR (95%CI) = 1.99(1.46-2.74) for mothers).

3.1.5 Factors associated with progress of insomnia in parents

The risk factors associated with incidence and persistence of insomnia in adults were

shown in table 9. Adults at age of 35-45 years old have lower incidence of insomnia than younger adults aged < 35 years (AOR(95%CI) = 0.50(0.35-0.87)) but similar incidence rate was found between those adults aged over 45 years and those aged below 35 years (AOR(95%CI) = 0.96(0.53-1.75), p>0.05). Unemployment at baseline could increase the incidence of insomnia with an AOR(95%CI) =1.67(1.04-2.70). Although children's behavioral problems (hyperactivity and frequent temper outbursts), nightmare, unwilling to get up in the morning, morning dry mouth, fatigue at baseline were correlated with incidence of insomnia in adults in univariate analysis, they could not be recruited into logistic regression after adjusting for age and gender. Among those day/night symptoms at baseline, only unrefreshness upon waking up and morning headache could predict incidence of insomnia in the final model. Females had higher persistence rate of insomnia than males with an AOR(95%CI) = 2.02(1.11-3.68). Subjects with lower educational level had a higher persistence rate of insomnia with an AOR(95%CI) of 2.78(1.09-7.17). Among those day/night symptoms at baseline, only morning headache predicted the persistence of insomnia in middle-aged adults. In view of potential confounding effects among the associated factors in relation with incidence/persistence of insomnia, all statistically significant associated factors were further tested by logistic regression model with forward likehood after controlling for age and gender. In this model, incidence of insomnia

could be predicted by unrefreshness upon waking up (AOR(95%CI) = 2.11(1.06-4.20)) and morning headache (AOR(95%CI) =5.31(1.50-18.8)) in the final model. Persistence of insomnia could predicted by fatigue but not education level and morning headache ($p>0.05$).

Table 9 Factors associated with progress of insomnia in adults

	Incidence		Persistence	
	Crude OR(95%CI)	**Adjusted OR(95%CI)**	**Crude OR(95%CI)**	**Adjusted OR(95%CI)**
Family information				
Gender				
Female vs Male	1.28(0.91-1.80)	0.98(0.60-1.61)	**2.11(1.31-3.42) ***	**2.02(1.11-3.68)***
Age				
35-45 years vs <=35 years	**0.53(0.34-0.85) ***	**0.50(0.35-0.87) ***	0.83(0.43-1.62)	---
>45 years vs <=35 years	0.87(0.49-1.51)	0.96(0.53-1.75)	0.64(0.27-1.48)	---
Noisy home				
Yes vs No	1.32(0.89-1.94)	---	1.51(0.91-2.50)	---
Education				
Non-tertiary level vs Tertiary level	1.12(0.70-1.82)	---	**3.03(1.28-7.14) ***	**2.78(1.09-7.17) ***
Occupation				
Unemployed vs Employed	**1.59(1.11-2.27) ***	**1.67(1.04-2.70) ***	0.65(0.40-1.05)	--
Marital status				
Married vs Non-married	0.65(0.41-1.03)	---	0.65(0.33-1.30)	---
Housing type				
Public vs Non-public	1.16(0.83-1.64)	---	1.64(1.04-2.58)	---
Family income				
>15,000 vs≤ 15,000 (HK$)	0.82(0.58-1.16)	---	0.76(0.48-1.22)	---
Nightmare ≥ 3 times/week				
Yes vs No	**3.85(1.27-11.66) ***	---	**2.52(1.18-5.41) ***	---
Snoring ≥ 3 times/week				
Yes vs No	1.29(0.89-1.88)	---	0.80(0.49-1.30)	---
Mouth breathing during sleep ≥ 3 times/week				
Yes vs No	1.11(0.63-1.97)	---	1.10(0.63-1.90)	---
Nocturnal sweating ≥ 3 times/week				

Yes vs No	1.62(0.57-3.59)	---	1.04(0.37-2.94)	---
Unwilling to get up in the morning ≥ 3 times/week				
Yes vs No	**1.61(1.04-2.47) ***	---	1.56(0.97-2.49)	---
Unrefreshness after awakening ≥ 3 times/week				
Yes vs No	**3.57(2.18-5.86) ***	**3.08(1.76-5.39) ***	**2.05(1.24-3.38) ***	---
Morning dry mouth ≥ 3 times/week				
Yes vs No	**2.26(1.44-3.54) ***	---	1.19(0.73-1.94)	---
Morning headache ≥ 3 times/week				
Yes vs No	**5.89(2.27-15.29) ***	**3.23(1.06-9.82) ***	**3.50(1.70-7.22) ***	**2.98(1.32-6.75) ***
Fatigue ≥ 3 times/week				
Yes vs No	**2.61(1.66-4.12) ***	---	**2.10(1.31-3.35) ***	**1.93(1.11-3.35) ***
Drink tea				
Often vs Not often	0.98(0.69-1.38)	---	0.99(0.62-1.57)	---
Drink coffee				
Often vs Not often	1.11(0.72-1.71)	---	0.77(0.44-1.36)	---
Drink wine				
Often vs Not often	1.18(0.61-2.30)	---	0.61(0.21-1.73)	---
Smoking				
Often vs Not often	1.27(0.75-2.15)	---	1.67(0.85-3.31)	---
Chronic use of medicine				
Often vs Not often	0.85(0.44-1.64)	---	1.65(0.93-2.93)	---
Children's insomnia at baseline				
Often vs Not often	1.90(0.89-4.04)	---	0.88(0.41-1.90)	---

Adjusted OR: Adjusted for age and gender

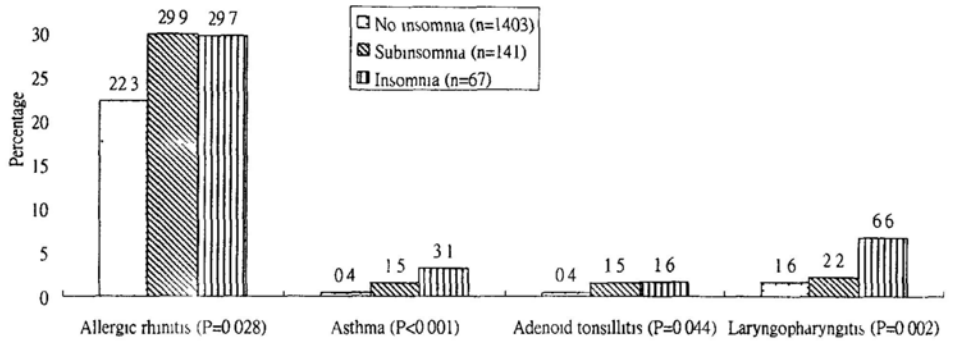
-- Without significance in adjusted model

* $p<0.05$

3.1.6 Major medical consequences of insomnia at 5-year follow-up in adolescents

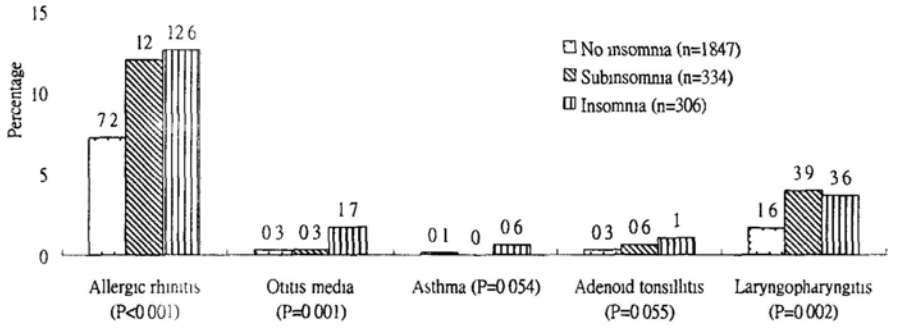
Figure 4 depicts that the relationship between insomnia frequencies at baseline and medical conditions at follow-up in adolescents (children). Insomnia frequency was positively associated with frequent episodes of upper airway inflammatory diseases, such as allergic rhinitis, asthma, adenoid-tonsillitis, and laryngopharyngitis but not nasosinusitis and otitis media during the past one year of the study. These associations persisted even after controlling for age, gender, family income, parental occupation and education level. Table 10 reports that those children with insomnia were 1.77 times more likely to report poor health condition than those children without insomnia symptoms (less than once/week). The AOR(95%CI) for frequent episodes of allergic rhinitis, asthma, laryngopharygngitis and chronic use of medicine in insomniac adolescents were 2.02(1.11-3.68), 19.82(2.91-134.93), 5.24(1.62-16.91), and 2.68(1.00-7.21) when compared with non-insomniac children respectively.

Figure 4 Insomnia as a predictor for self-reported health in adolescents



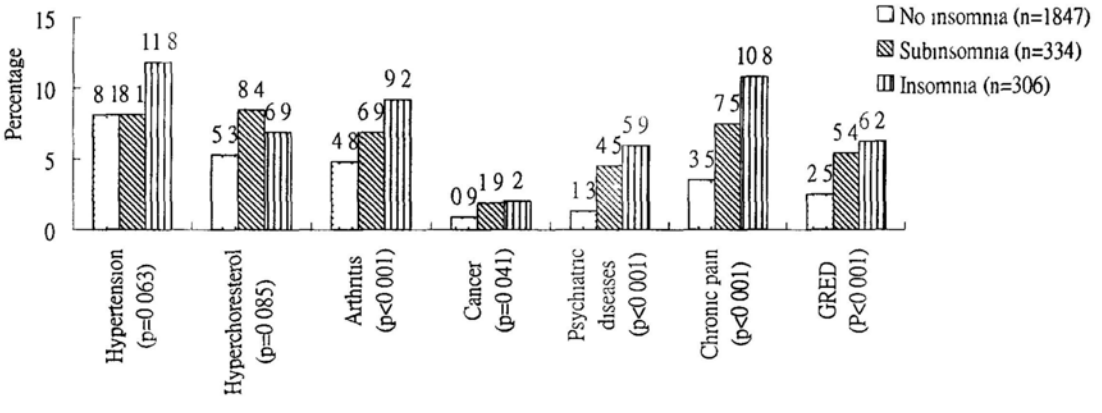
P for linear-by-linear association

Figure 5 Insomnia as a predictor for self-reported health in middle-age adults



P for linear-by-linear association

Figure 6 Insomnia as a predictor for chronic medical conditions in middle-age adults



P for linear-by-linear association

Table 10 Association between insomnia and major medical diseases in adolescents

	Crude OR(95%CI)	Model 1 Adjusted OR(95%CI)	Model 2 Adjusted OR(95%CI)
Self-reported poor health condition			
1-2 times/ week vs <1 time/week	---	---	---
≥ 3times vs <1 time/week	1.73(1.04-2.86) *	---	**1.79(1.01-3.17) ***
Allergic rhinitis			
1-2 times/ week vs <1 time/week	**1.49(1.01-2.19) ***	1.47(1.00-2.17) *	**1.64(1.09-2.46) ***
≥ 3times vs <1 time/week	1.47(0.85-2.55)	1.49(0.85-2.58)	**2.02(1.11-3.68) ***
Nasosinusitis			
1-2 times/ week vs <1 time/week	5.10(0.46-56.58)	---	---
≥ 3times vs <1 time/week	---	---	---
Otitis media			
1-2 times/ week vs <1 time/week	10.29(0.64-165.39)	---	---
≥ 3times vs <1 time/week	---	---	---
Asthma			
1-2 times/ week vs <1 time/week	4.05(0.78-21.10)	---	---
≥ 3times vs <1 time/week	**8.76(1.67-46.05) ***	**9.21(1.72-49.25) ***	**19.8(2.91-134.9) ***
Adenoid tonsillitis			
1-2 times/ week vs <1 time/week	4.06(0.78-21.14)	---	---
≥ 3times vs <1 time/week	4.39(0.51-38.15)	---	---
Laryngopharygngitis			
1-2 times/ week vs <1 time/week	1.37(0.41-4.65)	---	---
≥ 3times vs <1 time/week	**4.09(1.37-12.24) ***	**3.62(1.20-10.92) ***	**5.24(1.62-16.91) ***
Chronic use of medication			
1-2 times/ week vs <1 time/week	1.15(0.51-2.56)	---	---
≥ 3times vs <1 time/week	**2.68(1.17-6.13) ***	2.62(1.14-6.01) *	**2.68(1.00-7.21) ***

Model 1 adjusted for age and gender

Model 2 adjusted for age, gender, parental education, parental occupation, and family income

* $p<0.05$

-- Without significance in adjusted model

3.1.7 Major medical consequences of insomnia at 5-year follow-up in middle-aged adults

Figures 5 and 6 depict dose-response relationship between insomnia frequencies and major medical diseases in adults. Subjects were divided into 3 groups based on their insomnia frequencies (at least 3 times/week (insomnia), 1-2 times/week (subthreshold insomnia), and less than 1 time/week (no insomnia)). Linear-by-linear association analysis showed that frequency of insomnia symptoms at baseline could predict frequent episodes of allergic rhinitis, otitis media and laryngopharyngitis ($p<0.05$) and there were trends that insomnia frequency was associated with frequent episodes of asthma and adenoid tonsillitis ($p<0.10$) at 5 years follow-up. Frequency of insomnia symptoms at baseline was associated with self-reported arthritis, cancer, psychiatric disorders, chronic pain, and GERD ($p<0.05$) and there was a trend that insomnia was associated with hypertension (trend for $p=0.063$). We further combined those subjects without insomnia and subthreshold insomnia to compare with those subjects with insomnia frequency of at least 3 times/week and found that those subjects with insomnia at least 3 times /week had a higher prevalence of hypertension than those subjects with insomnia < 3 times/week ($p=0.033$, ARR(95%CI)=1.51(1.03-2.21)). Multivariate analysis between insomnia and other medical conditions are shown in

table 11. After controlling for age, gender, education, occupation, and family income as well as chronic use of medication at baseline, most of the associations of insomnia with medical conditions persisted except the association with cancer.

Table 11 reports that there are significant gender differences in prevalence (OR(95%CI) =1.65(1.26-2.15)) and persistence OR(95%CI)=2.45(1.10-4.23)) of insomnia at follow-up but not in incidence of insomnia (p>0.05). When age and current psychiatric disorders were recruited into the model, the gender differences in prevalence (OR from 1.65 to 1.51) and persistence (OR from 2.45 to 2.16) of insomnia were attenuated but remained significant.

Table 11 Association between insomnia at baseline and major medical diseases in middle-aged adults

	Incidence of medical conditions		
	Crude OR(95%CI)	**Model 1 Adjusted OR(95%CI)**	**Model 2 Adjusted OR(95%CI)**
Self-reported health condition			
1-2 times/ week vs <1 time/week	**2.23(1.55-3.21)***	**2.11(1.42-3.12) ***	**2.29(1.52-3.44) ***
≥ 3times vs <1 time/week	**2.38(1.66-3.42) ***	**2.11(1.41-3.16) ***	**2.54(1.68-3.84) ***
Allergic rhinitis			
1-2 times/ week vs <1 time/week	**1.76(1.23-2.51) ***	**1.69(1.16-2.47) ***	**1.59(1.08-2.34) ***
≥ 3times vs <1 time/week	**1.85(1.30-2.64) ***	**1.78(1.21-2.61) ***	**1.78(1.20-2.64) ***
Nasosinusitis			
1-2 times/ week vs <1 time/week	2.92(0.88-9.75)	---	---
≥ 3times vs <1 time/week	0.76(0.09-6.07)	---	---
Otitis media			
1-2 times/ week vs <1 time/week	0.97(0.12-8.05)	---	---
≥ 3times vs <1 time/week	6.15(1.97-19.18)	---	---
Asthma			
1-2 times/ week vs <1 time/week	--	---	---
≥ 3times vs <1 time/week	6.09(0.86-43.39)	---	---
Adenoid tonsillitis			
1-2 times/ week vs <1 time/week	3.50(0.83-14.71)	---	---
≥ 3times vs <1 time/week	3.66(0.87-15.39)	---	---
Laryngopharygngitis			
1-2 times/ week vs <1 time/week	**2.52(1.33-4.75)***	**2.63(1.35-5.13) ***	**2.97(1.50-5.89) ***
≥ 3times vs <1 time/week	**2.25(1.15-4.40) ***	**2.38(1.17-4.85) ***	**2.59(1.23-5.48) ***
Chronic use of medication			
1-2 times/ week vs <1 time/week	1.30(0.98-1.73)	**1.36(1.01-1.83) ***	1.34(0.99-1.82)
≥ 3times vs <1 time/week	**2.14(1.65-2.77) ***	**2.02(1.52-2.68) ***	**1.94(1.44-2.61) ***
Have you been diagnosed and received drug treatment for Hypertension			
1-2 times/ week vs <1 time/week	1.01(0.67-1.52)	---	---
≥ 3times vs <1 time/week	**1.55(1.08-2.23) ***	**1.54(1.04-2.29) ***	**1.73(1.16-2.59)***

Eye diseases			
1-2 times/ week vs <1 time/week	1.73(0.99-3.01)	---	---
≥ 3times vs <1 time/week	1.05(0.53-2.07)	---	---
Hypercholesterol			
1-2 times/ week vs <1 time/week	**1.69(1.12-2.56) ***	---	---
≥ 3times vs <1 time/week	1.35(0.85-2.13)	---	---
Arthritis			
1-2 times/ week vs <1 time/week	1.42(0.90-2.25)	---	---
≥ 3times vs <1 time/week	**2.11(1.40-3.18) ***	**1.96(1.24-3.08) ***	**2.05(1.28-3.29) ***
Epilepsy			
1-2 times/ week vs <1 time/week	---	---	---
≥ 3times vs <1 time/week	---	---	---
Cardiovascular diseases			
1-2 times/ week vs <1 time/week	2.13(0.94-4.83)	---	---
≥ 3times vs <1 time/week	1.10(0.38-3.22)	---	---
Cancer			
1-2 times/ week vs <1 time/week	2.05(0.86-4.88)	---	---
≥ 3times vs <1 time/week	2.15(0.90-5.11)	---	---
Diabetes mellitus			
1-2 times/ week vs <1 time/week	1.52(0.84-2.77)	---	---
≥ 3times vs <1 time/week	0.56(0.22-1.40)	---	---
Chronic lung diseases			
1-2 times/ week vs <1 time/week	---	---	---
≥ 3times vs <1 time/week	---	---	---
Eczema			
1-2 times/ week vs <1 time/week	0.88(0.54-1.45)	---	---
≥ 3times vs <1 time/week	1.08(0.68-1.73)	---	---
Psychiatric disorders			
1-2 times/ week vs <1 time/week	**3.08(1.67-5.70) ***	**3.58(1.86-6.91) ***	**3.24(1.61-6.49) ***
≥ 3times vs <1 time/week	**4.09(2.30-7.26) ***	**4.60(2.50-8.59) ***	**4.19(2.15-8.19) ***
Renal diseases			
1-2 times/ week vs <1 time/week	0.83(0.19-3.66)	---	---
≥ 3times vs <1 time/week	1.30(0.37-4.55)	---	---
Chronic pain			
1-2 times/ week vs <1 time/week	**2.29(1.46-3.60) ***	**2.30(1.42-3.72) ***	**2.46(1.51-4.01) ***

≥ 3times vs <1 time/week	**3.27(2.17-4.94) ***	**3.30(2.13-5.15) ***	**3.62(2.30-5.71) ***
GERD			
1-2 times/ week vs <1 time/week	**2.25(1.34-3.76) ***	**2.33(1.33-4.08) ***	**2.31(1.30-4.11) ***
≥ 3times vs <1 time/week	**2.24(1.33-3.78) ***	**2.57(1.47-4.52) ***	**2.80(1.59-4.94) ***

Model 1 adjusted for age and gender

Model 2 adjusted for age, gender, education, occupation, and family income as well as chronic use of medication at baseline.

* $p<0.05$; -- Without significance in adjusted model

Table 12 Gender differences between prevalence and persistence of insomnia at follow-up in adults

	Prevalence of insomnia		**Incidence of insomnia**		**Persistence of insomnia**	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Gender	**1.65**	**1.51**	1.39	1.19	**2.45**	**2.16**
	(1.26-2.15) *	**(1.05-2.18) ***	(0.95-2.02)	(0.71-1.98)	**(1.45-4.14) ***	**(1.10-4.23)***
Age	--	0.80	--	**0.61**	--	1.01
		(0.57-1.11)		**(0.39-0.94)***		(0.94-1.09)
Current psychiatric disorders	--	**5.88**	--	**10.6**	--	1.82
		(2.98-11.59) *		**(4.45-25.07)***		(0.52-6.42)

Model 1: univariate analysis; model 2 recruited gender, age and psychiatric disorders as independent variables.

* $p<0.05$

-- variables were not recruited into model 2 to predict dependent variables

3.1.8 Pubertal status and insomnia in adolescents

Figure 7 shows that there were no differences in the prevalence of overall insomnia and any subtypes of insomnia across pubertal status in adolescents ($p>0.05$). Furthermore, we could not find gender differences in prevalence of insomnia across different pubertal status ($p>0.05$) (figure 8).

Figure 7: Relationship between prevalence of insomnia and puberty (tanner stage)

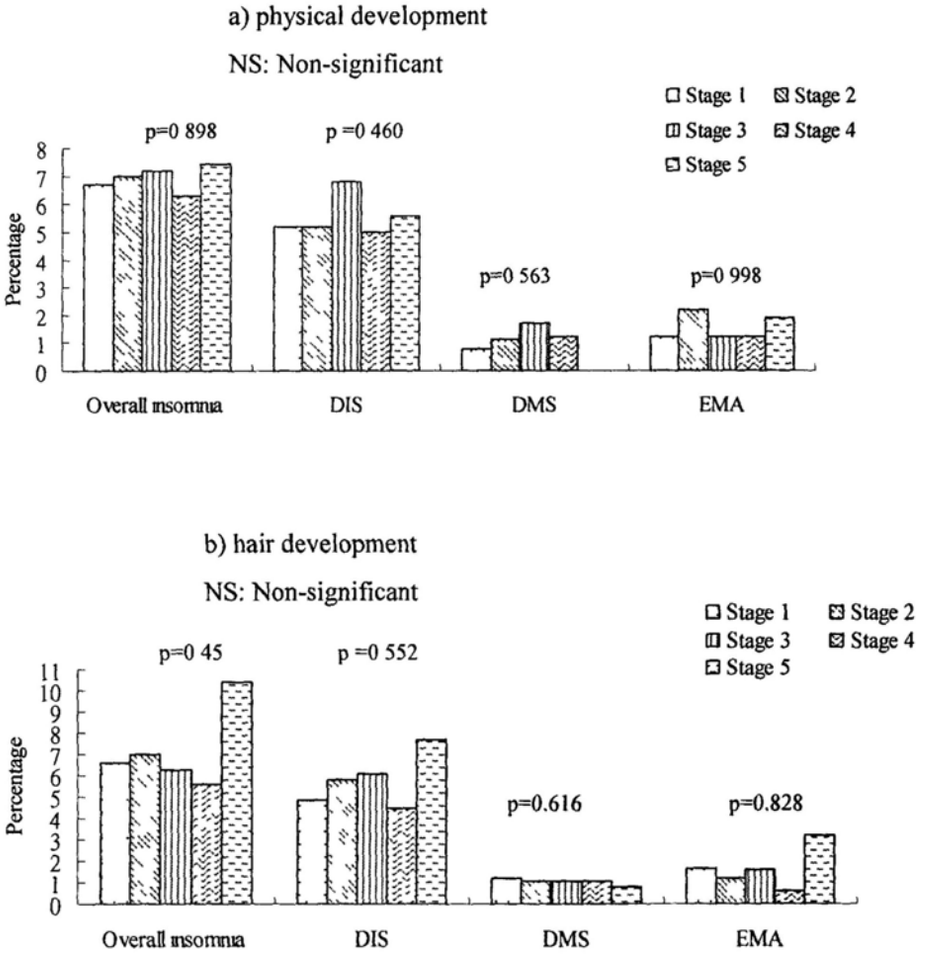
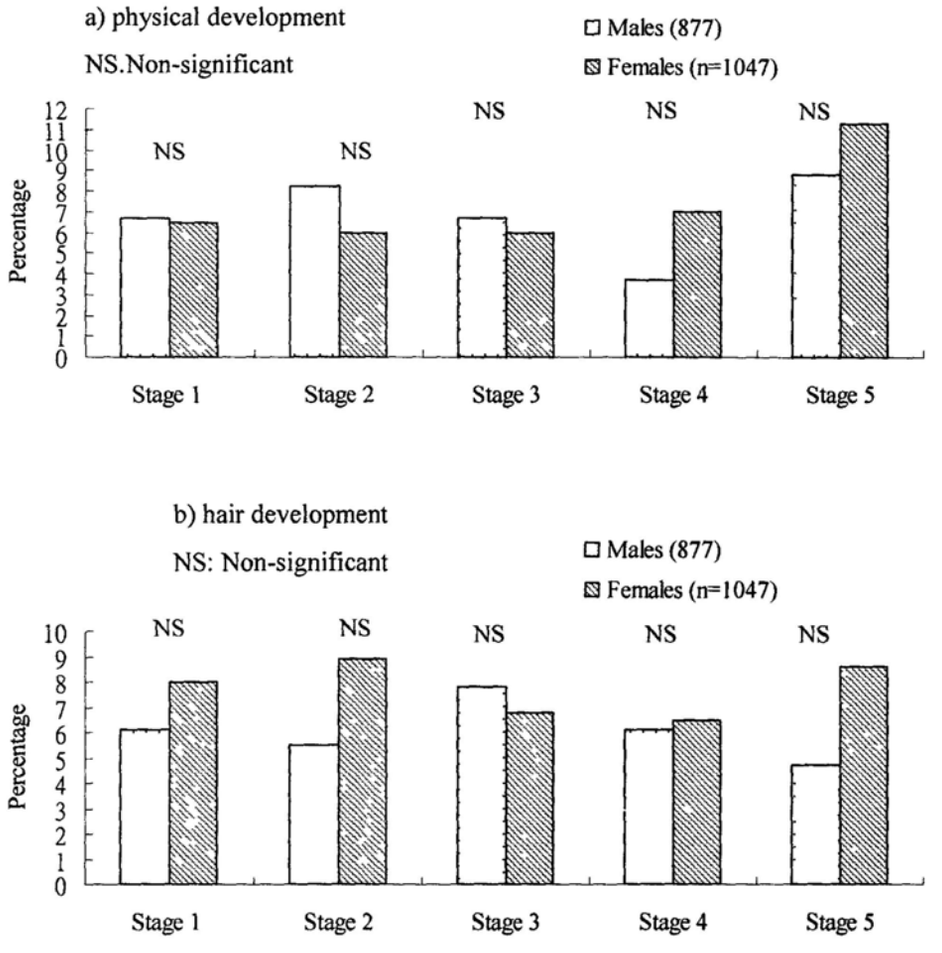


Figure 8 Gender differences across different stages of puberty in rate of insomnia



3.2 RESULTS FOR PHASE TWO STUDY

3.2.1 Sample characteristics in phase 2

Figure 2 reports the sample recruitment of the probands and their first degree relatives. A total of 75 adolescents were identified as insomniacs (insomniac proband) and 161 adolescents were non-insomniac control (non-insomniac proband) by phase 2 clinical interview (table 13). These adolescents together with their parents consist of 225 mother-offspring pairs, 198 father-offspring pairs, and 142 sibling pairs. Table 13 shows that insomniac adolescents were slightly older when compared to non-insomniac adolescents (14.1±1.8 vs 13.7±1.7, p=0.02). No differences in other socio-demographics were found between insomniac probands and control probands. There was no difference in socio-demographics between insomniacs and non-insomniacs in both mother and father groups. No age difference were found in sibling group between insomniacs and non-insomniacs (14.2±2.4 vs 14.5±4.1 years, p=0.698).

Table 13 Sample characteristics in phase 2 study (based on proband)

	Total sample	Insomniacs N=75	Non-insomniacs n=161	P value
age (years)*	13.9±1.8	14.4±1.8	13.8±1.7	0.020
Gender, male %	49.2%	52.8%	41.3%	0.101
Father occupation (employed),%	97.6%	97.2%	98.4%	0.634
Father educational level, tertiary or above, %	25.3%	22.2%	26.8%	0.464
Mother occupation (employed) , %	52.5%	53.2%	50.8%	0.757
Maternal educational level, tertiary or above, %	16.7%	11.1%	19.4	0.122
Housing type (public), %	42.8%	43.1%	42.7%	0.957
Family income > HK$15,000/month, %	73.5%	71.8%	74.2%	0.709

* $p<0.05$

3.2.2 Validity of questionnaire in the diagnosis of insomnia

Table 14 shows that questionnaire had high specificity (89.8%-95.7%) but rather low sensitivity (18.4%-37.5%) in predicting a clinical diagnosis of insomnia across different age groups. In other words, the questionnaires had mild to moderate agreements with clinical interview, with Kappa coefficients ranged from 0.18 to 0.31 and raw agreements ranged from 71.4% to 78.3%. Overall, the questionnaires had NPV of 50.0-78.5% and PPV of 58.3-82.6% for a clinical diagnosis of insomnia across different groups.

Table14 Accuracy of questionnaire in detection of insomnia when compared with clinical interview across different groups

	Sensitivity	**Specificity**	**NPV**	**PPV**	**Kappa coefficient**	**Raw agreement**
Proband, n=229	37.5%	89.8%	75.4%	62.8%	0.307	73.4%
Mothers, n=175	22.4%	95.7%	71.3%	72.2%	0.220	71.4%
Fathers, n=150	18.4%	95.5%	78.5%	58.3%	0.180	76%
Siblings, n=152	30.5%	91.6%	50.0%	82.6%	0.255	78.3%

*7 families had missing data in phase 1

NPV: negative predictive value PPV: positive predictive value

3.2.3 Familial aggregation of insomnia disorder

Table 15 shows that there was a robust familial aggregation phenomenon in insomnia. The parents of insomniac adolescents had higher rates of insomnia (both lifetime and current) when compared with those non-insomniac adolescents. There was a stronger maternal than paternal association. There was a trend that the siblings of insomniacs had higher insomniac rate than those siblings of non-insomniacs, albeit it did not achieve statistical significance ($p=0.106$ and 0.116 for current insomnia and lifetime insomnia respectively). This parent-offspring association in insomnia persisted even after controlling for the comorbid psychiatric disorders. The first degree relatives' recurrent rate was higher in those adolescents with insomnia than those adolescents without insomnia (43.9% vs 22.9% for current insomnia and 51.1% vs 28.0% for lifetime insomnia, respectively $p<0.001$). The familial aggregation of insomnia persisted in both logistic regression and generalized estimating equation (GEE) analysis.

Table 15 Familial aggregation of insomnia

	Insomniacs (n=75)	Controls (n=161)	P value¶	Adjusted OR(95%CI) &	Adjusted OR(95%CI)#
Rate of paternal insomnia (current), n(%)	21(35.0%)	30(22.1)	0.057	1.75 (0.88-3.47)	2.05 (1.04-4.04) *
Rate of paternal insomnia (lifetime), n(%)	24(40.0%)	31(22.8)	0.013	2.15 (1.10-4.21)*	2.45 (1.26-4.76)*
Rate of maternal insomnia (current), n(%)	37(52.9%)	41(26.8%)	<0.001	3.30 (1.77-6.14) ***	3.24 (1.75-5.99) ***
Rate of maternal insomnia (lifetime), n(%)	51(72.9%)	56(36.6%)	<0.001	5.00 (2.56-9.77) ***	5.24 (2.76-10.05) ***
Rate of sibling insomnia (current), n(%)	15(30%)	20(18.5%)	0.106	2.05 (0.92-4.55)	1.66 (0.74-3.74)
Rate of sibling insomnia (lifetime), n(%)	17(34%)	24(22.2%)	0.116	1.87 (0.88-4.01)	1.60 (0.74-3.46)
Recurrent rate of first degree relatives (current)	73(41.7%)	91(22.9%)	<0.001	2.74 (1.70-4.40)***	2.33 (1.57-3.48) ***
Recurrent rate of first degree relatives (lifetime)	92(51.1%)	111(28.0%)	<0.001	3.22 (2.03-5.10) ***	2.82 (1.94-4.08) ***

¶P value for Chi Square. & Logistic regression model: Adjusted for age, proband's gender and psychiatric disorders as well as paternal/maternal/sibling psychiatric disorders. # Generalized estimating equation model: Adjusted for age, proband's gender and psychiatric disorders as well as paternal/maternal/sibling psychiatric disorders.

*p<0.05; **p<0.01; ***p<0.001

3.2.4 Heritability of insomnia disorder

Table 16 shows that there was no significant association between father and mother in both current and lifetime insomnia, which suggested that insomnia was more like to be genetically transmitted in view of the strong association between parents and their children. Heritability analysis by SOLAR program suggested that the heritabilities were 0.57 ± 0.19 for current insomnia and 0.67 ± 0.13 for lifetime insomnia after adjusting for age and gender. In other words, the results suggested that 57% of variance of current insomnia and 67% of variance of lifetime insomnia could be accounted by the genetic variance. As insomnia is highly comorbid with psychiatric disorders as shown in our study, we excluded those subjects with current/lifetime psychiatric disorders for analysis of heritability of current/lifetime primary insomnia. The results as run by SOLAR program showed that there were also significant heritability for both current and lifetime primary insomnia with slight decrease in heritability estimation, for current primary insomnia was 0.45 ± 0.17 (p=0.007) and for lifetime primary insomnia was 0.58 ± 0.21 (p=0.004) respectively.

Table 16 Heritability for insomnia

	Proband-Mother pairs N =224	Proband-Father pair N =197	Proband-Sibling pair N=114	Father-mother pair N=150	Heritability[@] ± SE#	Heritability[&] ± SE#
Current Insomnia (OR(95%CI))	3.06 (1.70-5.53)	1.90 (0.98-3.71)	1.74 (0.66-4.60)	1.85 (0.86-3.98)	0.57 ± 0.19*	0.45 ± 0.17
Lifetime insomnia (OR(95%CI))	4.65 (2.50-8.65)	2.08 (1.09-3.98)	1.61 (0.66-3.95)	1.20 (0.60-2.46)	0.67 ± 0.13*	0.58 ± 0.21

adjusted for age and gender, *p<0.001

[@] Overall insomnia; [&] Primary insomnia

3.2.5 Interaction between parental history of insomnia and life stress on insomnia of offsprings

Table 17 shows the association of offspring's insomnia with life stress and parental history of insomnia. The probands and their siblings were grouped together in the analysis. Those adolescents with scores of Adolescent Self-Rating Life Events Check List (ASLEC) over 36 were defined to have high life stress (over 75 percentile). Finally, 90 (26.4%) out of 341 with completed data on ASLEC were defined as high life stress group and the others were defined as low life stress group. The adolescents with high life stress were 3.13 times more likely to have insomnia diagnosis than those with low life stress. The parental history of insomnia had a dose-response effect on the rate of insomnia in their offspring. Those adolescents with a single parent carrying a diagnosis of life time insomnia were 2.85 times more likely to have insomnia than those with neither parent having a history of insomnia, while the risk would increase to 4.97 times in those adolescents with both parents having histories of insomnia. Table 18 and figure 9 further show that there was significant interaction between parental history of insomnia and life stress on insomnia of adolescents ($p=0.002$). The risk difference between high and low life stress groups was similar in single parent with insomnia (16.4% vs 17.4%), while the risk difference in high life

stress group was 2.18 times higher (45.3% vs 20.8%, P<0.05) in those subjects with both parents having histories of insomnia than those with low life stress.

Table 17 Association of adolescents' insomnia with life stress and parental history of insomnia

	Insomniac adolescents	Non-insomniac adolescents	OR (95%CI)	P values
Life events N=341				
Low life stress N= 251	53(21.1%)	198 (78.9%)	Reference	Reference
High life stress N=90	41 (45.6%)	49(54.4%)	3.13(1.87-5.23)	<0.001
Parental history of insomnia (n=333)				
Neither parent with insomnia, n=135	20(14.8%)	115(85.2%)	Reference	Reference
Single parent with insomnia, n=157	52(33.1%)	105(66.9%)	2.85(1.60-5.08)	<0.001
Both parents with insomnia, n=41	19(46.3%)	22(53.7%)	4.97(2.29-10.79)	<0.001

Figure 9 Interaction between life stress and parental history of insomnia on adolescents' insomnia

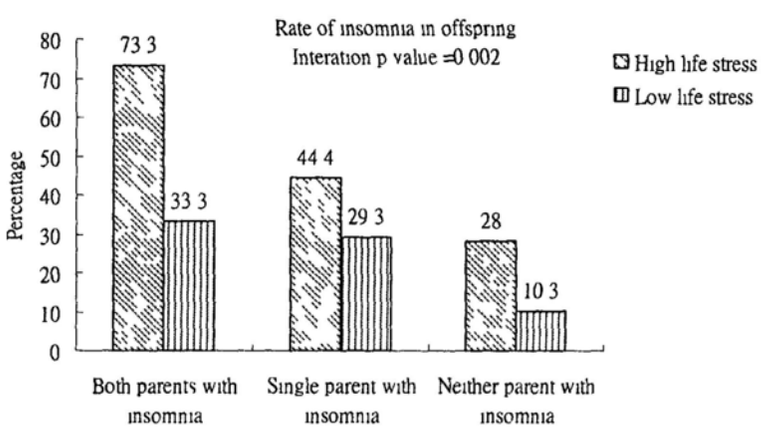


Table 18 Interaction between parental history of insomnia and life stress in risk of insomnia among adolescents

	Total N	Insomniac adolescents, n(%)	OR (95% CI)	Risk difference (95% CI)
Low life stress, N=244				
Neither parent with insomnia	107	12(10.3%)	Reference	Reference
Single parent with insomnia	112	32(28.6%)	3.17(1.53-6.55)*	17.4% (7.1%-21.8%)
Both parents with insomnia	25	8(32.0%)	3.73(1.33-10.47)*	20.8% (1.56%-40.0%)
High life stress, N=85				
Neither parent with insomnia,	25	7(28.0%)	Reference	Reference
Single parent with insomnia	45	20(44.4%)	2.06(0.72-5.89)	16.4% (-6.4%-39.2%)
Both parents with insomnia	15	11(73.3%)	7.07(1.68-29.83)*	45.3% (18.8%-73.8%)

* $P<0.05$

3.2.6 Association between insomnia and psychiatric disorders across different groups

As previous studies including our phase 1 study suggested that insomnia is highly comorbid with other psychiatric disorders, we further analyzed the association between insomnia and psychiatric disorders as defined by DSM-IV criteria according to clinical interviews. Figures 10 and 11 report the rates of psychiatric disorders between insomniacs and non-insomniacs across different groups. Among the 4 groups, significant comorbidity between insomnia and current psychiatric disorders could only be found in mother group with an OR (95%CI) =2.81(1.17-6.74), p=0.017). When combined all subjects into analysis, we found significant association between current insomnia and psychiatric disorders in all subjects (table 19). In addition, the lifetime insomnia was associated with both current and lifetime psychiatric disorders as well as mood and anxiety disorders (table 20).

Figure 10 Relationship between insomnia and psychiatric disorders

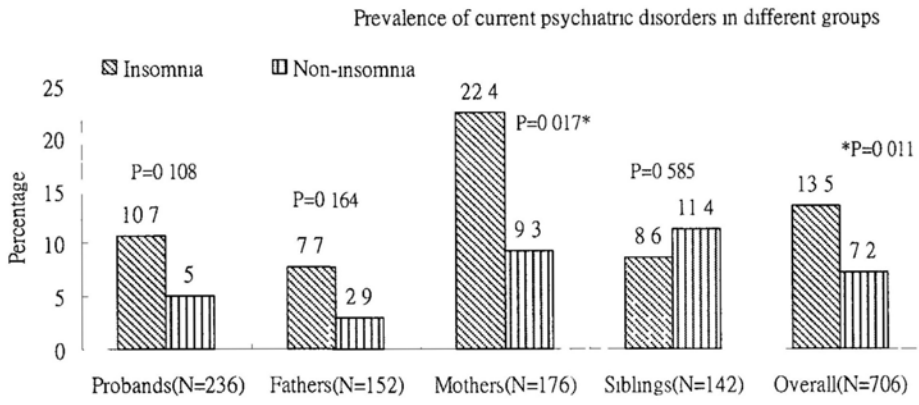
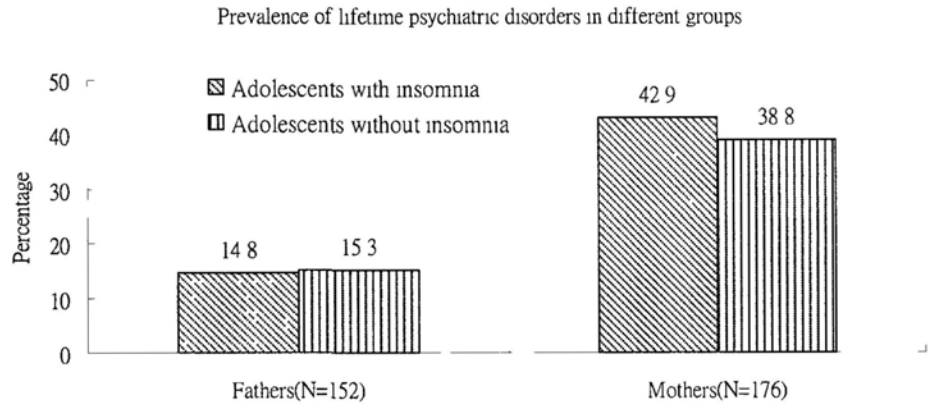


Figure 11 Prevalence of lifetime psychiatric disorders in different groups



Lifetime DSM-IV diagnosis was not available in adolescents and their siblings as Chinese version of DISC-IV does not provide lifetime assessment

Table 19 Association between current insomnia and psychiatric disorders in all subjects (probands, sibings and parents)

	Current psychiatric disorders	Lifetime psychiatric disorders	Mood disorders	Anxiety disorder	Other psychiatric disorders
Insomniacs, n=193	13.9%	26.7%	16.6%	9.8%	3.1%
Non-insomniacs, n=477	6.7%	16.9%	9.8%	6.3%	2.3%
Chi square	8.31	9.33	6.01	2.59	0.363
P value	0.004	0.002	0.014	0.108	0.547
OR (95% CI)	2.25 (1.28-3.94) *	1.90 (1.25-2.88) *	1.82 (1.12-2.96) *	1.63 (0.89-2.97)	1.36 (0.50-3.74)
Adjusted OR (95% CI) #	2.20 (1.26-3.82)*	1.67 (1.08-2.59) *	---	---	---

adjusted for age and gender; *p<0.05

-- No statistical significance found in adusted model

Mood disorders included major depressive disorder and bipolar disorder. Anxiety disorders included generalized anxiety disorder, social anxiety disorder, panic disorder, and anglophobia. Other psychiatric disorders included substance abuse, schizophrenia, eating disorder and attention deficit hyperactivity disorder.

Table 20 Association between lifetime insomnia and psychiatric disorders in all subjects

	Current psychiatric disorders	Lifetime psychiatric disorders	Lifetime mood disorders	Lifetime anxiety disorders	Lifetime other psychiatric disorders
Lifetime insomniacs, n=226	14.6%	30.1%	20.4%	11.5%	4.0%
Lifetime non-insomniacs, n=444	5.6%	12.4%	7.4%	5.2%	1.8%
Chi square	15.24	31.30	24.15	8.89	2.90
P value	<0.001	<0.001	<0.001	0.003	0.089
OR (95% CI)	2.87 (1.66-4.95) *	3.04 (2.04-4.54) *	3.19 (1.97-5.16) *	2.39 (1.33-4.29) *	2.27 (0.86-5.95)
Adjusted OR (95% CI) #	2.58 (1.48-4.51) *	2.44 (1.60-3.74) *	2.42 (1.44-4.09) *	2.16 (1.19-3.92) *	---

adjusted for age and gender; *p<0.05

-- No statistical significance found in adusted model

Mood disorders included major depressive disorder and bipolar disorder. Anxiety disorders included generalized anxiety disorder, social anxiety disorder, panic disorder, and anglophobia. Other psychiatric included substance abuse, schizophrenia, eating disorder and attention deficit hyperactivity disorder.

3.2.7 Could adolescents' insomnia predict first degree relatives' psychiatric disorders?

Table 21 reports the rate of psychiatric disorders in first degree relatives of insomniac probands and non-insomniac probands. The first degree relatives of insomniac probands had significantly higher risk of anxiety disorders than those of non-insomniac probands (11.9% vs 6.5%) with an OR (95% CI) = 1.93(1.04-3.58). We further tested the whether there was co-aggregation phenomenon between insomnia and anxiety. However, this association could not be maintained after adjusting for insomnia in first degree relatives and proband's anxiety disorders (p>0.05).

Table 21 Relationship between proband's insomnia and first degree relative's lifetime psychiatric disorders

	Overall psychiatric disorders	Mood disorders	Anxiety disorders	Other psychiatric disorders
First degree relatives of insomniacs, n=168	26.9%	17.9%	11.9%	2.4%
First degree relatives of non-insomniacs, n=367	23.2%	17.7%	6.5%	2.5%
Chi square	0.89	0.00	4.36	0.00
P value	0.34	0.97	0.036	0.96
OR (95% CI)	1.22(0.80-1.86)	1.01(0.63-1.63)	1.93(1.04-3.58)*	0.97(0.29-3.20)

*p<0.05

3.2.8 Heritability of psychiatric disorders

Table 22 shows the heritability of DSM-IV axis I psychiatric disorders. The lifetime psychiatric disorders had significant heritability with h^2 ±SE = 0.34±0.18, which indicated that psychiatric disorders were heritable in these Chinese families but there was no statistical significance in heritability for individual psychiatric disorder. No genetic correlations were found between insomnia disorder and psychiatric disorders when performing bivariate genetic analysis in SOLAR. The statistically negative results may be due to limited cases with psychiatric disorders in adolescent group.

Table 22 Heritability of DSM-IV axis I psychiatric disorders

	Current psychiatric disorders	Lifetime psychiatric disorders	Mood disorders	Anxiety disorders	Other psychiatric disorders
Sibling not recruited	0±0.50	0.30±0.25	0.50±0.38	0.19±0.33	0±0.50
Sibling recruited	0.15±0.21	0.34±0.18*	0.46±0.28#	0.26±0.22	0.22±0.42

* P<0.05; # p=0.053

All models were adjusted for age and gender

3.2.9 Comparison of insomnia severity, sleep quality and mood symptoms between insomniacs and non-insomniacs in adolescents and middle-aged adults

Similar results were found between insomniac adolescents and non-insomniac adolescents as well as between insomniac adults and non-insomniac adults in various psychometric measures (table 23). The insomniacs in both groups had higher scores in ISI (9.7±4.1 vs 4.8±3.8 in adolescents and 11.8±4.2 vs 6.9±4.4 in adults, $p<0.001$), PSQI (7.4±2.7 vs 3.9±2.4 in adolescents and 9.0±3.2 vs 5.2±2.6 in adults, $p<0.001$), FIRST (20.1±5.3 vs 17.7±5.6 in adolescents and 24.0±9.6 vs 20.7±5.8 in adults, $p<0.001$), MEQ (12.6±3.5 vs 14.0±3.2 in adolescents and 14.9±3.6 vs 16.1±3.0 in adults, $p<0.01$), BDI (5.4±5.0 vs 3.3±4.3 in adolescents and 5.5±5.2 vs 3.1±3.8 in adults, $p=0.001$), and HADS anxiety score (6.1 ± 3.5 vs 4.3 ± 3.3 in adolescents and 5.8±3.8 vs 4.3±3.6 in adults, $p<0.01$) than their non-insomniac counterparts. Insomniac adults had higher score in DBAS than non-insomniac adults (89.0 ± 9.5 vs 85.1 ± 9.6, $p<0.001$) and there was a near-significant trend that insomniac adolescents had a higher score in DBAS than non-insomniac adolescents (82.4 ±15.3 vs 79.2 ± 12.5, $p=0.052$).

3.2.10 Comparison of pain and other somatic symptoms between insomniacs and non-insomniacs

Table 24 shows that insomniacs had higher scores of pain over pervasive areas and higher scores in somatic symptoms than non-insomniacs in both adolescent and adult groups. Table 25 further shows that there were higher scores in VAS headache (17.5±20.5 vs 10.1±15.4, $p<0.001$), SSI non-pain score (33.7±11.4 vs 31.0±96.4, $p=0.041$) and SSI total score (47.0±15.1 vs 43.4±13.0, $p=0.039$) among mothers than fathers. No gender differences were found in VAS pain scores and somatic scores between female and male adolescents ($p>0.05$). Tables 26-29 further show that the severity of insomnia was correlated with poor outcomes of various psychometric measures of pain and somatic symptoms.

Table 23 Insomnia diagnosis, insomnia severity, sleep quality, and mood symptom

	Adolescents		P values	Parents		P values
	Insomniacs N=94	Non-insomniacs N=248		Insomniacs N=97	Non-insomniacs N=229	
ISI	9 7±4 1	4 8±3 8	<0 001*	11 8±4 2	6 9±4 4	<0 001*
PSQI	7 4±2 7	3 9±2 4	<0 001*	9 0±3 2	5 2±2 6	<0 001*
DBAS	82 4±15 3	79 2±12 5	0 052	89 0±9 5	85 1±9 6	0 001*
FIRST	20 1±5 3	17 7±5 6	<0 001*	24 0±9 6	20 7±5 8	<0 001*
MEQ	12 6±3 5	14 0±3 2	0 001*	14 9±3 6	16 1±3 0	0 005*
BDI	5 4±5 0	3 3±4 3	0 001*	5 5±5 2	3 1±3 8	0 001*
HADS-anxiety	6 1±3 5	4 3±3 3	<0 001*	5 8±3 8	4 3±3 6	0 003*

*Adjusted significant P value was < 0.025 (adjusted for multiple comparisons).

Adjusted for age and gender by ANCOVA

Table 24 Insomnia diagnosis, pain and other somatic symptoms

	Adolescents		P values	Parents		P values
	Insomniacs N=69	Non-insomniacs N=190		Insomniacs N=78	Non-insomniacs N=178	
VAS (overall pain)#	22 7±22 9	14 2±17 6	0 003*	32 8±23 6	19 8±20 5	<0 001
VAS (headache) #	18 0±24 3	12 6±18 4	0 083	19 3±22 3	11 9±16 5	0 018*
VAS (backache) #	16 8±2 4	9 3±17 0	0 012*	29 5±25 2	15 2±19 8	<0 001
VAS (shoulder and neck pain) #	21 7±25 8	10 9±17 6	0 001*	25 5±24 1	18 5±21 9	0 016*
VAS (daily pain) #	11 4±16 9	8 2±15 5	0 016*	21 0±21 6	13 8±18 2	0 003*
VAS (wakeup pain#)	19 2±23 2	10 5±16 5	0 008*	28 5±26 2	17 1±18 4	0 003*
SSI (pain)	12 0±4 4	10 1±3 7	0 001*	14 7±4 4	12 1±4 1	<0 001*
SSI (non-pain)	32 7±11 1	28 4±8 8	0 001*	36 7±11 7	30 5±9 4	<0 001*
SSI total score	44 8±14 9	38 5±12 0	0 001*	51 4±15 4	42 7±12 7	<0 001*

variables were tested by Wilcoxon signed rank test Other comparisons were tested by paired-samples *t* test ,*Adjusted significant P value was < 0 025 (adjusted for multiple comparisons)

Adjusted for age and gender by ANCOVA

3.2.11 Gender and insomnia-interaction effects on pain and somatic symptoms

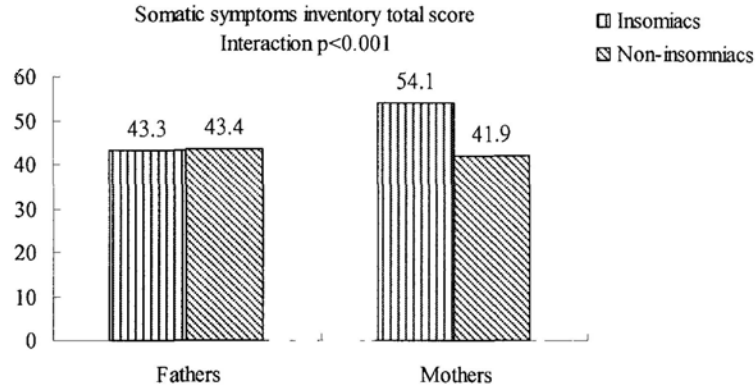
As there were gender differences in some pain and somatic symptoms, we further performed two-way ANOVA to analyze whether there were gender and insomnia interaction effects on pain and somatic symptoms. Figure 12 (a-c) shows significant interaction effects between insomnia and gender on SSI pain score, non-pain scale and total score ($p<0.05$). Insomnia could lead to more pain and other somatic complaints in females but not males. There was also a trend in interaction effects on pain and somatic symptoms between insomnia diagnosis and genders in adolescents groups ($p=0.063$-0.095) (data not shown)

Table 25 Gender differences in pain and other somatic symptoms

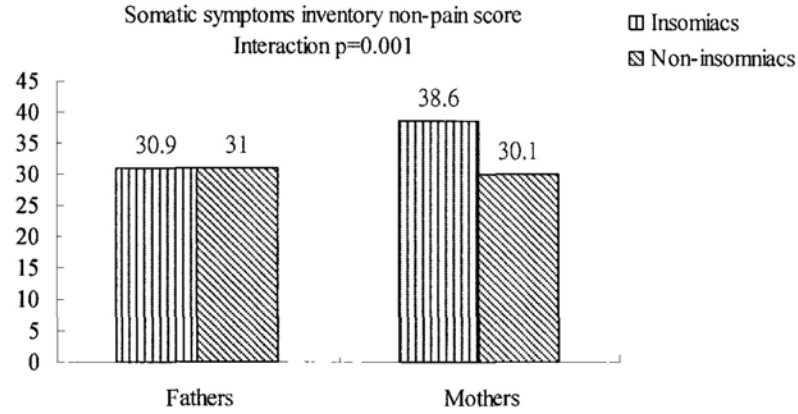
	Adolescents		P values	Parents		P values
	Females N=146	Males N=121		Mothers N=143	Fathers N=118	
VAS (overall pain)#	16 2±18 3	16 5±20 4	0 903	25 6±22 0	22 1±22 9	0 097
VAS (headache) #	12 5±17 6	15 7±22 7	0 709	17 5±20 5	10 1±15 4	0 001*
VAS (backache) #	11 8±19 7	10 3±17 3	0 487	20 9±22 5	17 9±22 6	0 110
VAS (shoulder and neck pain) #	15 8±23 0	11 9±18 0	0 083	22 4±23 1	18 5±22 4	0 124
VAS (daily pain) #	8 5±15 0	10 3±17 3	0 676	16 3±19 2	15 6±19 9	0 385
VAS (wakeup pain) #	12 3±17 8	13 2±19 7	0 416	21 7±21 4	19 9±22 6	0 225
SSI (pain)	10 6±3 8	10 6±4 1	0 933	13 3±4 5	12 4±4 0	0 095
SSI (non-pain)	29 5±9 3	29 3±9 9	0 871	33 7±11 4	31 0±96 4	0 041*
SSI total score	40 1±12 5	39 9±13 6	0 885	47 0±15 1	43 4±13 0	0 039*

Variables were tested by Wilcoxon signed rank test Other comparisons were tested by paired-samples *t* test * $p<0$ 05

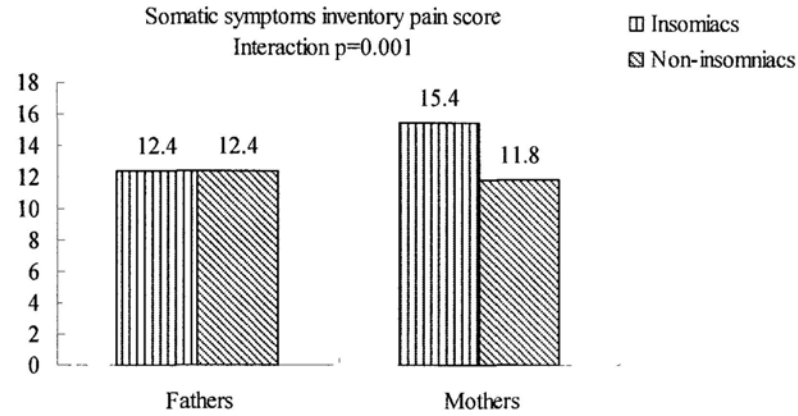
Figure 12 Gender and insomnia diagnosis interaction on somatic symptoms in middle-aged adults



a)



b)



c)

Table 26 Relationship of insomnia severity with pain and somatic symptoms in adolescents

	ISI (adolescents)		PSQI (adolescents)	
	All sample N=263	**Insomniacs N=68**	**All sample N=263**	**Insomniacs N=68**
VAS (overall pain)#	0 286**	0 112	0 227**	0 388**
VAS (headache) #	0 292**	0 173	0 236**	0 392**
VAS (backache) #	0 245**	0 131	0 180**	0 388**
VAS (shoulder and neck pain) #	0 275**	0 110	0 240**	0 355**
VAS (daily pain) #	0 312**	0 153	0 218**	0 295**
VAS (wakeup pain#)	0 271**	0 024	0 247**	0 313**
SSI (pain)	0 396**	0 169	0 404**	0 542**
SSI (non-pain)	0 466**	0 367**	0 429**	0 586**
SSI total score	0 462**	0 323**	0 437**	0 597**

Variables were tested by Spearman correlation analysis Others were tested by Pearson correlation analysis *Adjusted significant P value was < 0 025 (adjusted for multiple comparisons).

Table 27 Relationship of insomnia severity with pain and somatic symptoms in adults

	ISI (adults)		PSQI (adults)	
	All sample N= 243	**Insomniacs N=77**	**All sample N= 243**	**Insomniacs N=77**
VAS (overall pain)#	0 343**	0 299**	0 362**	0 191
VAS (headache) #	0 215**	0 178	0 192**	0 016
VAS (backache) #	0 260**	0 137	0 284**	0 061
VAS (shoulder and neck pain) #	0 312**	0 220	0 245**	-0 017
VAS (daily pain) #	0 391**	0 258*	0 373**	0 136
VAS (wakeup pain#)	0 303**	0 177	0 325**	0 075
SSI (pain)	0 403**	0 356**	0 385**	0 287*
SSI (non-pain)	0 507**	0 545**	0 480**	0 510**
SSI total score	0 501**	0 515**	0 476**	0 469**

variables were tested by Spearman correlation analysis. Others were tested by Pearson correlation analysis *Adjusted significant P value was < 0.025 (adjusted for multiple comparisons).

Table 28 Linear regression analysis between ISI, SSI and BDI and anxiety

	Adults		Adolescents	
	B±SE	**P value**	**B±SE**	**P value**
Age	0.30±0.18	0.099	0.12±0.21	0.582
Gender, male	-2.5±1.5	0.101	-0.43±1.24	0.727
ISI	0.69±0.17	<0.001*	0.70±0.16	<0.001*
BDI	1.02±0.23	<0.001*	1.17±0.18	<0.001*
HADS anxiety	0.67±0.28	0.015*	0.44±0.24	0.062

*Adjusted significant P value was < 0.025 (adjusted for multiple comparisons).

SSI as dependent variable

Table 29 Linear regression analysis between ISI, SSI-pain and BDI and anxiety

	Adults		Adolescents	
	B±SE	**P value**	**B±SE**	**P value**
Age	0.08±0.06	0.166	0.05 ± 0.07	0.491
Gender, male	-0.64±0.50	0.203	-0.11±0.40	0.782
ISI	0.20±0.06	<0.001*	0.17±0.05	0.001*
BDI	0.28±0.08	<0.001*	0.32±0.06	<0.001*
HADS anxiety	0.16±0.10	0.086	0.11±0.08	0.143

*Adjusted significant P value was < 0.025 (adjusted for multiple comparisons).

SSI-pain as dependent variable

3.2.12 Personality differences between insomniacs and non-insomniacs across different groups

Table 30 shows the five dimensions of personality as based on NEO-FFI scale between insomniacs and non-insomniacs across different groups. Higher scores in neuroticism (24.4±7.7 vs 20.2±7.1 for adolescent and 20.6±5.7 vs 18.1±6.0 for adult respectively, $p<0.001$) and lower scores in extraversion (27.6±6.9 vs 29.8±5.9 for adolescent and 23.8±5.0 vs 25.3±4.8 for adult respectively, $p<0.025$) were found in insomniac than non-insomniac adolescents and adutls. Furthermore, insomniac adolescents had lower scores in agreeableness (26.9±5.3 vs 28.7±4.7, $p=0.003$) and conscientiousness (26.1±5.8 vs 28.4±5.1, $p=0.001$) than non-insomniac adolescents.

Table 30 Comparison of personality between insomniacs and non-insomniacs

	N	E	O	A	C
Adolescent group					
Insomniacs (n=92)	24.4±7.7	27.6±6.9	26.2±4.8	26.9±5.3	26.1±5.8
Non-insomniacs (n=243)	20.2±7.1	29.8±5.9	25.6±4.1	28.7±4.7	28.4±5.1
P value	<0.001*	0.008*	0.216	0.003*	0.001*
Adult group					
Insomniacs (n=92)	20.6±5.7	23.8±5.0	24.8±4.0	30.9±3.9	31.5±4.5
Non-insomniacs (n=222)	18.1±6.0	25.3±4.8	25.0±3.8	30.9±4.1	32.0±4.3
P value	<0.001*	0.015*	0.068	0.909	0.355

*Adjusted significant P value was < 0.025 (adjusted for multiple comparisons).

Adjusted for age and gender by ANCOVA; N: Neuroticism; E: Extraversion; O: Openness; A: Agreeableness; C: Conscientiousness

3.2.13 Impact of personality on subjective severity of insomnia in insomniacs

As previous results suggested that insomnia diagnosis was correlated with several aspects of personality, we further analyzed whether the subjective severity of insomnia as measured by ISI and PSQI was associated with specific personality. Due to the limited cases of insomnia in sibling group (n=21) and father group (n=39), insomniac probands and their insomniac siblings were grouped together and so were insomniac mothers and insomniac fathers. There were no differences in ISI, PSQI, personality scores and SF-36 total and subscale scores between insomniac probands and insomniac siblings and also between insomniac fathers and mothers, which further supported that the combinations would not lead to further bias. Subjective insomnia severity as indicated by ISI scores and subjective sleep quality as indicated by PSQI were positively correlated with neuroticism across different groups ($p<0.05$) (table 30). Middle-aged insomniacs with lower extraversion in personality tended to report more severe insomniac symptoms and poorer sleep quality $r=-0.235$ ($p<0.05$) while insomniac adolescents with higher conscientiousness score reported lesser severity of insomnia symptoms (table 31).

Table 31 Correlation between personality and subjective insomnia severity in insomniacs across different groups

	N	E	O	A	C
Adolescent insomniacs (n=94)					
ISI	0 265**	-0 050	0 057	0 041	-0 230*
PSQI	0 414**	-0 046	0 093	0 080	-0 087
Adults insomniacs (n=97)					
ISI	0 460**	-0 235*	-0 297*	0 062	-0 064
PSQI	0 327**	0 104	-0 231*	0 117	-0 144

Adolescent group included both insomniac probandsand their affected siblings

*Adjusted significant P value was < 0 025 (adjusted for multiple comparisons) **p<0 01

Adjusted for age and gender by ANCOVA, N Neuroticism, E Extraversion, O Openness, A Agreeableness, C Conscientiousness

3.2.14 Comparison of health related QoL between insomniacs and non-insomniacs across different groups

As the demography is very similar in probands and their siblings, we combined these 2 groups together to analyze the differences in health related quality of life (QoL) between insomniacs and non-insomniacs. Table 32 shows that insomniacs in all groups reported poor overall QoL (71.3±11.9 vs 76.8±11.9 in adolescents group, 66.9±11.7 vs 72.3±12.4 in mother group, and 67.9±14.7 vs 75.1±11.5 in father group, $p<0.05$), poor emotional well being (68.0±16.3 vs 75.3±14.9 in adolescent group, 68.5±15.1 vs 75.1±13.1in mother group, and 67.9±14.7 vs 75.1±11.5 in father group, respectively; $p<0.05$). However, there were no differences in physical functioning, role limitations due to physical health, and energy/fatigue between insomniacs and non-insomniacs in any groups. Insomniac adolescents reported more social functioning impairment than non-insomniac adolescents (79.0±18.4 vs 84.2±16.2, $p=0.012$) but there were no differences in social functioning between insomniacs and non-insomniacs in both mother and father groups. Insomniac adolescents and mothers were more likely to report poor pain-related QoL than their non-insomniac counterparts (75.6±20.0 vs 82.7±19.0 in adolescent group and 65.6±22.5 vs 71.5±22.7 in mother group, respectively; $p<0.05$).

Table 32 Comparisons in health related QoL between insomniacs and non-insomniacs

	Non-insomniacs	Insomniacs	P value
Adolescent group	N=247	N=94	
Physical functioning	93 9±10 6	92 1±11 4	0 217
Role limitations due to physical health	83 9±18 7	79 9±18 9	0 084
Role limitations due to emotional problems	80 7±20 3	73 9±21 4	0 009*
Energy/fatigue	64 8±11 7	62 0±13 0	0 055
Emotional well being	75 3±14 9	68 0±16 3	<0 001*
Social functioning	84 2±16 2	79 0±18 4	0 016*
Pain	82 7±19 0	75 6±20 0	0 006*
General health	65 9±17 5	59 0±17 8	0 002*
Total score of SF-36	76 8±11 9	71 3±11 9	<0 002*
Adult group	N=229	N=97	
Physical functioning	88 3±22 9	83 7±17 3	0 160
Role limitations due to physical health	83 3±17 1	80 7±15 3	0 232
Role limitations due to emotional problems	82 3±17 4	79 7±17 7	0 181
Energy/fatigue	63 0±13 0	61 6±13 1	0 594
Emotional well being	74 2±13 8	68 6±14 0	<0 000*
Social functioning	79 7±17 7	75 1±17 7	0 014*
Pain	70 2±21 6	59 8±20 4	0 001*
General health	62 1±17 7	53 0±16 6	0 001*
Total score of SF-36	73 5±12 2	67 2±12 6	<0 001*

*Adjusted significant P value was < 0 025 (adjusted for multiple comparisons)

Adjusted for age and gender by ANCOVA

3.2.15 Impact of insomnia severity on health related QoL

Table 33 shows that consistent with our previous findings, physical functioning score in SF-36 was correlated with neither insomnia diagnosis nor insomnia severity in young and middle-aged insomniacs. Overall speaking, the severity of insomnia symptoms was correlated with pervasive aspects of QoL in both young and middle-aged insomniacs ($p<0.05$). Although there were no differences in role limitations due to physical health and energy/fatigue scores between insomniac adolescents and non-insomniac adolescents, these two subscales scores were negatively correlated with insomnia severity and sleep quality in correlation ($p<0.05$).

Table 33 Linear regression analysis between SF-36 and ISI

	Adults (ISI)		Adolescents (ISI)	
Dependent variables	B±SE	P value	B±SE	P value
Physical functioning	-0.32±0.27	0.238	0.09±0.17	0.614
Role limitations due to physical health	-0.85±0.23	<0.001*	-0.38±0.28	0.180
Role limitations due to emotional problems	-0.90±0.23	<0.001*	-0.70±0.30	0.022*
Energy/fatigue	-0.29±0.19	0.118	-0.13±0.19	0.513
Emotional well being	-0.62±0.14	<0.001*	-0.60±0.21	0.004*
Social functioning	-0.91±0.26	<0.001*	-0.62±0.25	0.014*
Pain	-1.26±0.29	<0.001*	-0.23±0.30	0.435
General health	-0.73±0.23	0.002*	-0.50±0.26	0.053
Total score of SF-36	-0.75±0.16	<0.001*	-0.44±0.17	0.010*

*Adjusted significant P value was < 0.025 (adjusted for multiple comparisons).

Adjusted for age, gender, BDI and HADS anxiety scores by ANCOVA

3.2.16 Modulation of personality trait on health related QoL in insomniacs across different groups

Table 34 shows that neuroticism was strongly and negatively correlated with all aspects of health related QoL in insomniac adolescents ($r=-0.220$-0.645; $p<0.05$). Except for role limitations due to physical health and pain subscale, similar association between neuroticism and other subscales scores and total score of QoL was also found in middle-aged insomniacs ($r=0.208$-0.669; $p<0.05$). Furthermore, extraversion was positively correlated with energy/fatigue, emotional well being, general health, and total score of SF-36 in insomniac adolescents ($r=0.293$-0.423; $p<0.05$) while extraversion was positively correlated with role limitations due to emotional problems, energy/fatigue, and emotional well being in insomniac adults ($r=0.207$-0.347; $p<0.05$). Openness and agreeableness were not correlated with any aspects of QoL in neither group. The insomniac adolescents with higher score in conscientiousness reported higher score in emotional well being ($r=0.27$, $p<0.05$).

Table 34 Correlation between personality and quality of life in insomniacs across different groups

	N	E	O	A	C
Adolescent group (n=94)					
Physical functioning	-0 277**	0 131	-0 090	-0 031	0 035
Role limitations due to physical health	-0 437**	-0 057	0 052	0 195	-0 035
Role limitations due to emotional problems	-0 438**	0 076	-0 232*	0 065	0 057
Energy/fatigue	-0 460**	0 380**	-0 068	-0 034	0 130
Emotional well being	-0 645**	0 408**	-0 157	0 170	0 270**
Social functioning	-0 256*	0 105	-0 125	0 148	-0 43
Pain	-0 220*	0 012	0 074	-0 095	0 050
General health	-0 444**	0 423**	0 041	0 206*	0 209*
Total score of SF-36	-0 561**	0 293**	-0 177	0 062	0 107
Parent group (n=97)					
Physical functioning	-0 237*	0 159	-0 052	0 129	0 005
Role limitations due to physical health	-0 085	0 078	0 071	0 123	-0 036
Role limitations due to emotional problems	-0 428**	0 207*	0 006	0 098	0 077
Energy/fatigue	-0 208*	0 347**	-0 040	0 010	-0 046
Emotional well being	-0 669**	0 310**	0 010	0 061	0 162
Social functioning	-0 421**	0 144	0 032	0 013	0 174
Pain	-0 136	-0 008	-0 025	0 017	0 000
General health	-0 380**	0 183	-0 089	0 040	-0 164
Total score of SF-36	-0 407**	0 142	0 038	0 040	0 051

Adolescent group included probands and their siblings while parent group included fathers and mothers

*Adjusted significant P value was < 0 025 (adjusted for multiple comparisons), **p<0 01

N Neuroticism, E Extraversion, O Openness, A Agreeableness, C Conscientiousness

3.2.17 Familial clustering and heritability in psychological distress

By this family study approach with father-mother-offspring trios, we could analyze the heritability of the phenotypes running in family. Table 35 illustrates the correlation coefficients between family members and heritabilities of various psychometric measures. The results indicated that serial psychological distress tended to run in family and were moderately heritable. Significant heritability was found in ISI (0.27±0.09), PSQI (0.30±0.11), HADS anxiety (0.38±0.11), SSI total score (0.42±0.11) and several aspects of QoL as well as VAS pain scores. When siblings were recruited into analysis to enlarge the sample size, significant heritability emerged in neuroticism, agreeableness and conscientiousness. No significant heritability was found in MEQ, DBAS, and BDI scores whether siblings were recruited into analysis or not.

Table 35 Familial correlations and heritability in psychometric assessments

	Proband-Mother pairs (n=215)	Proband-Father pairs (n=191)	Father-mother pairs (n=146)	Heritability ± SE
ISI	0 155*	0 143*	0 082	0 27±0 09***
PQSI	0 211**	0 057	-0 019	0 30±0 11**
DBAS	0 055	0 011	0 086	0±0 50
FIRST	0 085	0 023	0 103	0 10±0 09
MEQ	0 072	-0 057	0 049	0 03±0 09
Personality				
Neuroticism	0 106	0 031	0 077	0 11±0 09
Extraversion	0 035	0 013	-0 091	0 03±0 10
Openness	0 004	0 026	-0 070	0 01±0 10
Agreeableness	0 061	-0 093	-0 183*	0±0 50
Conscientiousness	0 020	0 007	0 093	0 06±0 06
SF-36				
Physical functioning	-0 048	0 087	0 072	0±0 50
Role limitations due to physical health	0 063	0 083	0 030	0 13±0 10
Role limitations due to emotional problems	0 079	0 047	-0 019	0 11±0 09
Energy/fatigue	0 273**	0 279**	0 154	0 48±0 08***
Emotional well being	0 061	0 045	0 148	0 09±0 09
Social functioning	0 150*	0 176*	0 208*	0 27±0 09**
Pain	0 149	-0 023	0 059	0 09±0 10
General health	0 091	0 172*	0 028	0 21±0 09*
Total score of SF-36	0 391**	0 165*	0 238**	0 41±0 09***
VAS (overall pain)	0 140	0 085	0 084	0 12±0 11
VAS (headache)	0 192*	0 195*	0 306**	0 30±0 10**
VAS (backache)	0 096	0 138	0 274**	0 19±0 10*
VAS (shoulder and neck pain)	0 192*	0 296**	0 314**	0 36±0 10***
VAS (daily pain)	0 094	0 122	0 202*	0 06±0 12
VAS (wakeup pain)	0 217**	0 088	0 040	0 22±0 11*
SSI (pain)	0 313**	0 028	0 169	0 37±0 11***
SSI (non-pain)	0 242**	0 150	0 134	0 42±0 11***
SSI total score	0 277**	0 123	0 170	0 42±0 11***
BDI	0 099	0 166*	-0 053	0 17±0 11
HADS anxiety	0 090	0 219*	0 072	0 38±0 11**

Heritability was adjusted for age and gender, *p<0 05, **p<0 01, *** p<0 001

3.2.18 Bivariate genetic analysis between insomnia severity and sleep quality and other psychometric measures

The above mentioned results suggested that insomnia and sleep quality was highly

correlated with other psychometric measures. Bivariate genetic analysis was employed to differentiate what proportion of the variances of these correlations could be accounted by the variances of genetic and environmental component. Table 36 reports the phenotypic correlations (ρ_P) between insomnia severity (as measured by ISI) and sleep quality (as measured by PSQI) and other psychometric measures as well as the proportions of these correlations attributable to genetic (ρ_G) and environmental (ρ_E) factors respectively. For phenotypic correlations, ISI and PSQI were correlated with all psychological distress, neuroticism and extraversion personality and all aspects of SF-36 with exception of physical functioning score. The correlation between ISI and HADS anxiety score was 0.40 after adjusting for age and gender ($p<0.01$). The variance of genetic component could account for 51±21% of the variance of the correlation between ISI and HADS anxiety score, while environmental component could account for 34±10% of variance of the correlation between ISI and HADS anxiety. These results indicated that the genetic component played a major role in the correlation between ISI and HADS anxiety score. The relationship between ISI and MEQ score was mainly due to environmental component rather than genetic component. Overall speaking, the phenotypic correlations between insomnia severity and sleep quality and other psychometric measures were due to both genetic and environment component. Genetic component played major role in the relationship of

insomnia severity and sleep quality with HADS anxiety score, BDI score, somatic symptom inventory score (mainly non-pain), neuroticism personality, and emotional wellbeing, social functioning, general health and total scores in SF-36; while environmental component played a major role in the relationship of insomnia severity and sleep quality with MEQ, extraversion personality, conscientiousness, FIRST, energy and pain scores in SF-36.

Table 36 Bivariate genetic analysis between insomnia severity and sleep quality and other psychometric measures

		ISI	PSQI
HADS anxiety	ρ_P	0.40**	0.35**
	ρ_G	0.51±0.21*	0.68±0.22**
	ρ_E	0.34±0.10**	0.19±0.12
BDI	ρ_P	0.44***	0.40**
	ρ_G	0.56±0.29*	0.87±0.31**
	ρ_E	0.41±0.09**	0.25±0.10**
MEQ	ρ_P	-0.23*	-0.26*
	ρ_G	-0.50±0.79	--
	ρ_E	-0.21±0.08**	-0.16±0.08*
SSI total	ρ_P	0.46**	0.44**
	ρ_G	0.63±0.18**	0.81±0.19**
	ρ_E	0.38±0.10**	0.25±0.11*
SSI non-pain	ρ_P	0.46**	0.43**
	ρ_G	0.70±0.18**	0.86±0.19**
	ρ_E	0.35±0.11**	0.21±0.12
SSI pain	ρ_P	0.40**	0.38*
	ρ_G	0.41±0.22*	0.63±0.21**
	ρ_E	0.40±0.10**	0.27±0.11*
Neuroticism	ρ_P	0.43**	0.37**
	ρ_G	0.53±0.27*	0.81±0.30**
	ρ_E	0.41±0.07**	0.27±0.09**
Extraversion	ρ_P	-0.20*	-0.20*
	ρ_G	0.65±0.81	-0.03±0.57
	ρ_E	-0.36±0.08**	-0.24±0.09**
Openness	ρ_P	-0.01	0.02
	ρ_G	--	--
	ρ_E	-0.09±0.09	-0.05±0.09
Agreeableness	ρ_P	-0.12	-0.02
	ρ_G	-0.52±0.43	0.04±0.36
	ρ_E	-0.03±0.09	-0.03±0.16
Conscientiousness	ρ_P	-0.19*	-0.16
	ρ_G	-0.32±0.68	-0.14±0.65
	ρ_E	-0.19±0.08*	-0.17±0.09

FIRST	p_P	0.42**	0.39**
	p_G	--	--
	p_E	0.28±0.07**	0.20±0.08*
SF-36			
physical functioning	p_P	-0.12	-0.11
	p_G	--	--
	p_E	0.09±0.10	0.03±0.10
Role limitations due to physical health	p_P	-0.25*	-0.26*
	p_G	--	-0.58±0.35
	p_E	-0.07±0.08	-0.19±0.09*
Role limitations due to emotional problems	p_P	-0.34**	-0.32**
	p_G	--	-0.97±0.45*
	p_E	0.14±0.07*	-0.18±0.09*
Energy/fatigue	p_P	-0.29**	-0.23*
	p_G	-0.05±0.19	0.01±0.20
	p_E	-0.44±0.09**	-0.39±0.10**
Emotional well being	p_P	-0.44**	0.38**
	p_G	-0.82±0.38*	--
	p_E	-0.38±0.07**	-0.25±0.07
Social functioning	p_P	-0.29**	-0.26**
	p_G	-0.65±0.23**	-0.31±0.24
	p_E	0.15±0.09	-0.24±0.09*
Pain	p_P	-0.35**	-0.31**
	p_G	--	--
	p_E	-0.20 ±0.07**	0.16±0.08*
General health	p_P	-0.36**	-0.39**
	p_G	-0.59 ±0.24**	-0.41±0.24
	p_E	-0.29±0.08**	-0.39±0.08**
Total score of SF-36	p_P	-0.43**	-0.41**
	p_G	-0.65 ± 0.15**	-0.58±0.16**
	p_E	-0.33±0.09**	-0.32±0.09**

-- p_G could not be computed. *p<0.05; **p<0.01

p_P: correlation between phenotypes; p_G: correlation accounted by genetic component; p_E: correlation accounted by genetic component

3.2.19 Comparison of heart rate variability (HRV) between insomniacs and non-insomniacs

Table 37 shows that there were no differences in heart rate variability between insomniacs and non-insomniacs in either adolescent groups or adult groups ($p>0.05$). Table 38 further shows the relationship between psychometric assessments and HRV variables. Consistent with results in table 37, insomnia severity (as measured by ISI) and sleep quality (as measured by PSQI) were not correlated with HRV variables in either adolescents or adults ($p>0.025$). FIRST score was, however, correlated with VLF ($r = -0.191$), HF ($r= 0.261$) and LF/HF ratio ($r = -0.183$) in adolescent group. However, only the statistically significant relationship between FIRST and HF persisted after adjusting for age and gender in linear regression model ($p=0.009$). No correlation between HRV variables and HADS anxiety score and SSI total score was found in either adolescents or adults.

Table 37 Comparison of daytime heart rate variability among adolescents and adults

	Adolescents			Parents		
	Insomniacs N=51	**Non-insomniacs N=152**	**P values**	**Insomniacs N=49**	**Non-insomniacs N=131**	**P values**
Mean RR (Hz)	0 90±0 14	0 88±0 15	0 417	0 95±0 13	0 97±0 12	0 156
RRSTD	0 08±0 03	0 07±0 03	0 339	0 04±0 02	0 04±0 02	0 687
RMSSD	78 3±42 9	72 7±39 9	0 404	36 8±17 9	36 1±24 0	0 695
VLF	25 9±14 1	28 8±15 5	0 763	31 5±13 9	34 1±15 5	0 429
LF	31 0±10 9	32 5±11 8	0 607	33 5±12 5	32 5±13 5	0 509
HF	43 3±18 7	38 7±17 7	0 503	35 1±15 9	33 3±19 4	0 833
LF/HF ratio	1 01±0 91	1 18±0 98	0 670	1 35±1 19	1 73±1 95	0 272

Adjusted for age and gender by ANCOVA

Table 38 Correlations between psychometric assessments and HRV in adolescents and adults

	ISI	**PSQI**	**FIRST**	**SSI**	**BDI**	**HADS-anxiety**
Adolescent group N=205						
Mean RR (Hz)	-0 029	-0 061	0 038	0 024	0 158	0 077
RRSTD	-0 036	-0 091	-0 022	-0 095	-0 053	0 083
RMSSD	0 018	0 003	0 104	-0 018	0 079	0 004
VLF	-0 112	-0 074	-0 191**	-0 032	-0 160	-0 107
LF	-0 002	-0 122	-0 147	-0 053	-0 095	-0 015
HF	0 100	0 142	0 261***	0 062	0 204**	0 102
LF/HF ratio	0 008	0 072	-0 183**	-0 040	-0 143	-0 003
Adult group N= 177						
Mean RR (Hz)	-0 131	-0 078	-0 129	-0 140	-0 167	-0 170
RRSTD	-0 099	-0 037	-0 013	-0 108	-0 114	-0 039
RMSSD	-0 139	-0 038	-0 023	-0 054	-0 131	-0 053
VLF	-0 023	-0 132	-0 042	-0 163	-0 010	-0 112
LF	0 162	0 112	0 082	0 016	0 091	0 121
HF	-0 097	0 026	-0 027	-0 122	-0 054	0 008
LF/HF ratio	0 075	-0 039	0 049	-0 097	0 007	0 089

* P<0.025 was considered as statistical significance in view of multiple comparison.

3.2.20 Familial clustering and heritability in HRV variables

Table 39 shows that mean RR had significant familial aggregation phenomenon (r=0.244-0.583, p<0.05). Furthermore, there were significant correlations in RRSTD (r=0.352, p<0.01) and RMSSD (r=0.537, p<0.01) between sibling pairs. Genetic analysis shows significant heritabilities in mean RR (h^2 ± SE = 0 41±0 12, P<0 001) and VLF (h^2 ± SE = 0.26±0.14, P<0.05) after adjusting for age and gender When siblings were recruited into genetic analysis, RRSTD and RMSSD also presented with significant heritabilities (p<0.05).

Table 39 Familial correlations and heritability in HRV variables

	Proband-Mother pairs (n=118)	Proband-Father pairs (n=101)	Proband-Sibling pairs (n=124)	Father-mother pairs (n=82)	Heritability ± SE	Heritability ± SE[£]
Mean RR	262**	0 280**	0 583**	0 244*	0 41±0 12***	0 40±0 09***
RRSTD	0 019	0 129	0 352**	0 022	0 08±0 13	0 22±0 10*
RMSSD	0 061	0 110	0 537**	0 159	0 13±0 12	0 31±0 09***
VLF	0 102	0 147	0 242	-0 104	0 26±0 14*	0 20±0 11*
LF	-0 052	0 066	0 055	-0 134	0 03±0 16	0±0 50
HF	0 041	0 075	0 224	-0 124	0 13±0 13	0 15±0 10
LF/HF ratio	-0 008	0 059	0 149	0 065	0±0 50	0 05±0 11

Heritability was adjusted for age and gender

[£]siblings were recruited into analysis

*p<0 05, **p<0 01, *** p<0 001

3.3 RESULTS FOR PHASE THREE STUDY

3.3.1 Sample characteristics of phase 3 study

A total of 167 probands were recruited into phase 3 study. Among them, 54 were diagnosed as insomniacs while the other 83 adolescents were non-insomniacs by clinical interview. A total of 130 mothers (42 current insomniac cases), 114 fathers (27 current insomniac cases) and 88 siblings (15 current insomnia cases) of these adolescents were also recruited in phase 3 study. This total sample consisted of 255 adolescents and 244 middle-aged adults.

3.3.2 Comparison of Objective and subjective sleep quality between insomniacs and non-insomniacs

Table 40 shows the differences in objective and subjective sleep parameters as measured by 3-day actigraphy and sleep questionnaires between insomniac adolescents and non-insomniac adolescents. For objective sleep parameters, insomniac adolescents had longer time in bed (537±63 min vs 518±57 mine, p=0.049) and actual sleep time (446±59 vs 423±53, p=0.008) than non-insomnia. While the

objective sleep quality as indicated by sleep efficiency, sleep onset latency and wake after sleep onset did not differ between insomniac adolescents and non-insomniac adolescents in actigraphy. For subjective assessments, insomniac adolescents had longer sleep onset latency (22±26 min vs 14±19 min, $p<0.001$) than non-insomniac adolescents. Table 42 shows that there were good agreements in time in bed and actual sleep time, low but significant correlation in sleep onset latency, but no correlation in wake after sleep onset and sleep efficiency between actigraphy and questionnaire in adolescents.

Tables 40 and 41 show the comparison results in objective and subjective sleep parameters as measured by actgraphy between insomniac and non-insomniac adults. Insomniac adults had longer WASO (47±31 min vs 39±23 min, $p=0.049$) than non-insomniac adults, but did not differ in TIB, AST, SOL and sleep efficiency from those of non-insomniac adults. For the subjective measures by questionnaire, insomniac adults had shorter TIB (442±60 min vs 455±60 min, $p=0.043$) and AST (388±65 min vs 429±70 min, $p<0.001$), longer SOL (29±22 min vs 16±16 min, $p<0.001$) and WASO (21.4±37.1 min vs 13.4±38.6 min, $p=0.021$), and lower sleep efficiency (88%±9% vs 94%±9%, $p=0.002$) than non-insomniac adults. Moderate to good correlation was found in TIB and AST ($r=0.794$ and $r=0.562$; respectively, $p<0.001$)

between actigraphy and questionnaire in middle-aged adults.

Table 40 Objective and subjective sleep quality as measured by 3-day actigraphy and questionnaires between insomniacs and non-insomniacs in adolescents

	Non-insomniacs	Insomniacs	P value for differences	
	N=133	N=74	Mean	Standard deviation
Actigraphy				
Time in bed, min	518±66	537±65	0.049*	0.341
Actual sleep time, min	423±53	446±59	0.008**	0.079
Sleep onset latency, min	19.9±14.3	20.0±15.4	0.953	0.944
Wake after sleep onset, min	54.0±24.3	50.6±28.7	0.332	0.923
Sleep efficiency, %	81.9±5.2	83.2±7.2	0.221	0.890
Questionnaire				
Time in bed, min	516±73	528±66	0.173	0.643
Actual sleep time, min	498±88	504±69	0.913	0.062
Sleep onset latency, min	14±19	22±26	<0.001**	0.176
Sleep efficiency, %	95±6	96±12	0.022*#	0.915
Wake after sleep onset, min	7.1±47.1	5.8±30.2	0.809	0.855

Adolescent group included probands and their siblings.*p<0.05; **p<0.01;

Non-parametric test for all variables.

insomnia group had lower sleep efficiency although the means of SE was higher in insomnia group.

Table 41 Objective and subjective sleep quality as measured by 3-day actigraphy and questionnaires between insomniacs and non-insomniacs in adults

	Non-insomniacs N=135	Insomniacs N=74	P value for differences	
			Mean	Standard deviation
Actigraphy				
Time in bed, min	456±62	454±81	0.828	0.323
Actual sleep time, min	379±60	373±69	0.518	0.192
Sleep onset latency, min	16±17	18±21	0.247	0.089
Wake after sleep onset, min	39±23	47±31	0.049*	0.022*
Sleep efficiency, %	83.9±8.5	83.7±14.5	0.116	0.257
Questionnaire				
Time in bed, min	455±60	442±60	0.043*	0.173
Actual sleep time, min	429±70	388±65	<0.001**	0.128
Sleep onset latency, min	16±16	29±22	<0.001**	0.256
Sleep efficiency, %	94±9	88±9	0.002**	0.178
Wake after sleep onset, min	13.4±38.6	21.4±37.1	0.021*	0.645

Adult group included fathers and mothers.*p<0.05; **p<0.01;

Non-parametric test for all variables.

Table 42 Correlations between objective and subjective assessments in sleep quality and quantity

	Time in bed	Actual sleep time	Sleep efficiency	WASO	SOL
Adults	0.844**	0.562**	0.112	0.010	0.031
Adolescents	0.933**	0.665**	0.051	0.067	0.245**

Nonparametric Spearman test (correlation coefficients)

*p<0.05; **p<0.01

3.3.3 Comparison of serial salivary cortisol profiles between insomniacs and non-insomniacs in both adults and adolescents

Tables 43 and 44 show the differences in serial salivary cortisol profiles between gender and insomnia status in both adolescents and adults. There were no differences in serial salivary cortisol profiles between insomniacs and non-insomniacs in adult groups after adjusting for age and gender by ANCOVA model. Male adults had higher cortisol than female adults at noon and afternoon (4 pm) ($p<0.05$). There were gender and insomnia interactions on evening (10 pm) cortisol level ($p=0.009$) and AUCi ($p<0.05$) in adult group, which shows that insomniac female had higher AUCi than non-insomniac female while insomniac male even had lower AUCi (AUCi2 and AUCi3) than non-insomniac males (please also see figure 13). Similar insomnia * gender interaction at 10 pm cortisol level was found ($p=0.011$). Insomniac females had lower cortisol level than non-insomniac females (1.7±1.0 nmol/L vs 2.5±1.9 nmol/L) while insomniac males had higher cortisol level than non-insomniac males (2.9±2.2 nmol/L vs 2.4±1.6). Similar results were found when those subjects with current psychiatric disorders as defined by SCID interview were excluded (data not showed).

However, no such kinds of gender differences were found in adolescents. Female adolescents had higher cortisol levels at 30 min, 60 min and 90 min after waking up

(p<0 05) as well as all aspects of AUCs related to CAR (p<0 05) There was no interaction between gender and insomnia on any cortisol profiles in adolescents groups Insomniac adolescents had lower cortisol at T1 (awakening) and higher AUCi3 than non-insomniac adolescents

Table 43 Comparison of serial salivary cortisol level between insomniacs and non-insomniacs in adolescents

	Non-insomniacs		Insomniacs		P insomnia	P gender	P interation
	Female N=87	Male N=89	Female N=33	Male N=39			
T1, nmol/L	10 3±4 8	10 9±5 6	9 9±3 8	8 6±4 3	0 040*	0 641	0 327
T2, nmol/L	12 6±7 1	10 5±5 9	13 5±7 4	9 1±4 6	0 730	0 001**	0 341
T3, nmol/L	8 3±4 5	6 8±4 5	9 8±6 4	6 6±3 3	0 398	0 001**	0 398
T4, nmol/L	5 8±2 9	5 1±3 6	7 3±5 3	5 4±3 2	0 105	0 015*	0 471
T5, nmol/L	4 3±2 8	4 1±3 1	3 7±2 7	3 5±3 3	0 160	0 130	0 579
T6, nmol/L	3 7±2 7	3 5±3 3	3 6±2 8	3 2±2 6	0 629	0 366	0 920
T7, nmol/L	2 0±1 3	1 9±1 3	2 1±1 5	1 9±1 5	0 857	0 245	0 978
AUCg1, nmol/L	22 8±9 9	21 4±9 5	23 3±9 2	17 7±6 9	0 184	0 008**	0 250
AUCg2, nmol/L	31 2±13 5	28 2±13 1	33 2±15 0	24 3±9 3	0 518	0 002**	0 256
AUCg3, nmol/L	36 9±15 4	33 3±15 7	40 4±19 2	29 7±11 4	0 871	0 001**	0 841
AUCi1, nmol/L	2 3±7 2	-0 3±6 4	3 6±7 3	0 5±5 5	0 250	0 003**	0 919
AUCi2, nmol/L	1 3±9 7	-2 4±8 8	3 6±10 2	-0 5±7 4	0 110	0 003**	0 967
AUCi3, nmol/L	-2 0±14 3	-7 2±13 8	2 3±14 8	-3 1±11 7	0 034*	0 007**	0 236
Cortisol slope 1	0 48±0 41	0 44±0 87	0 52±0 33	0 47±0 51	0 209	0 546	0 908
Cortisol slope 2	0 56±0 42	0 61±0 35	0 61±0 30	0 52±0 59	0 177	0 792	0 271
Cortisol slope 3	0 76±0 19	0 78±0 22	0 78±0 14	0 72±0 27	0 572	0 445	0 122

ANCOVA analysis adjusted for age and gender

*p<0 05, **p<0 01,

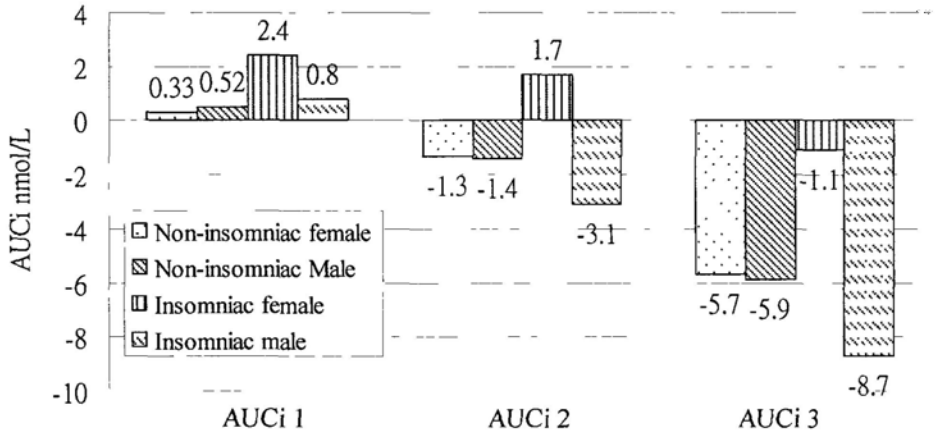
Table 44 Comparison in serial salivary cortisol levels between insomniacs and non-insomniacs in adults

	Non-insomniacs		Insomniacs		P insomnia	P gender	P interation
	Female N=85	Male N=89	Female N=47	Male N=29			
T1, nmol/L	11.6±5.7	12.0±4.9	10.8±4.9	13.3±6.8	0.990	0.278	0.076
T2, nmol/L	12.0±6.1	13.0±7.2	13.5±5.2	12.7±7.1	0.503	0.852	0.322
T3, nmol/L	8.5±4.9	8.2±4.9	9.3±4.3	9.1±4.4	0.186	0.942	0.924
T4, nmol/L	6.0±3.5	4.0±3.5	6.7±4.2	7.1±3.8	0.266	0.437	0.663
T5, nmol/L	4.5±3.0	5.6±3.5	4.3±2.2	5.6±3.7	0.865	0.002	0.038
T6, nmol/L	3.7±2.1	4.9±3.9	3.1±1.8	4.9±3.6	0.502	0.023	0.490
T7, nmol/L	2.5±1.9	2.4±1.6	1.7±1.0	2.9±2.2	0.573	0.065	0.011*
AUCg1, nmol/L	23.7±10.6	24.5±11.0	23.9±9.5	27.3±11.8	0.432	0.507	0.442
AUCg2, nmol/L	32.2±15.0	32.7±15.0	33.2±13.6	36.8±15.6	0.304	0.678	0.504
AUCg3, nmol/L	38.4±18.2	39.3±18.5	39.8±16.8	44.2±18.5	0.297	0.626	0.554
AUCi1, nmol/L	0.33±5.6	0.52±5.6	2.4±4.8	0.8±8.4	0.522	0.274	0.049*
AUCi2, nmol/L	-1.3±7.8	-1.4±7.4	1.7±6.3	-3.1±11.2	0.444	0.192	0.047*
AUCi3, nmol/L	-5.7±12.2	-5.9±11.4	-1.1±9.6	-8.7±16.1	0.456	0.179	0.043*
Cortisol slope 1	0.5±0.5	0.5±0.3	0.6±0.3	0.3±1.0	0.311	0.069	0.118
Cortisol slope 2	0.6±0.3	0.6±0.3	0.7±0.3	0.6±0.4	0.782	0.178	0.474
Cortisol slope 3	0.7±0.4	0.8±0.2	0.8±0.2	0.8±0.2	0.543	0.905	0.138

ANCOVA analysis adjusted for age and gender

*p<0.05; **p<0.01

Figure 13 Gender and insomnia diagnosis interaction on cortisol AUCi in adults



3.3.4 Correlation between salivary cortisol and 24-hour urinary cortisol

Table 45 shows the correlation between salivary cortisol and 24-hour urinary cortisol in both adults and adolescents. Lower correlations were found between these two sampling methods for assessment of cortisol in adults than in adolescents. In adolescents, 24-hour urinary cortisol was positively correlated with salivary cortisol levels at awakening, 30 min, 60 min and 90 min after awakening and 10 pm ($p<0.05$). Similar results were also found after adjusting for age and gender in linear regression model. The mild to moderate correlations or even no correlation between serial salivary cortisol and 24-hour urinary cortisol (especially in adults group) indicated that 24-hour urinary cortisol might reflect distinct aspect in HPA axis functioning.

Table 45 Correlation between salivary cortisol and 24-hour urinary cortisol

	Adolescents N=117	Adults N=129
T1 cortisol	0.308**	0.105
T2 cortisol	0.325***	0.184*
T3 cortisol	0.208*	0.168
T4 cortisol	0.190*	0.165
T5 cortisol	0.087	-0.023
T6 cortisol	0.128	0.182*
T7 cortisol	0.292**	0.197*
AUCg1, nmol/L	0.401***	0.169
AUCg2, nmol/L	0.362***	0.175*
AUCg3, nmol/L	0.345***	0.180*
AUCi1, nmol/L	0.074	0.099
AUCi2, nmol/L	0.025	0.084
AUCi3, nmol/L	0.029	0.065
Cortisol slope 1	0.092	0.059
Cortisol slope 2	0.047	-0.114
Cortisol slope 3	0.034	-0.184*

*p<0.025; **p<0.01

Similar results were found after adjusting for age and gender in linear regression model.

3.3.5 Familial clustering in cortisol profiles (serial salivary and 24-hour urinary cortisol)

Table 46 reports that there were correlations in each time point of salivary cortisol and AUCg of CAR between probands and their mothers ($r=0.172$-0.317, $p<0.05$). However, no correlation was found in diurnal slope and AUCi of CAR between proband and mothers. The proband-father correlations in salivary cortisol were mainly in latter part of daytime (from 90 min after awakening to 10 pm) with correlation coefficients ranging from 0.24 to 0.294. There were significant correlations in cortisol levels at 30 min and 60 min after awakening, noon time and 10 pm, AUCg of CAR and AUCi3 of CAR as well as diurnal slopes 1 and 3 between siblings. Significant correlations in cortisol profile were also found between spouses (awakening, 90 min after awakening, and all aspects of AUCg and AUCi of CAR). Genetic analysis by POLAR further showed that there were statistically significant heritabilities in each time point of salivary cortisol level (h^2 range from 0.28 to 0.45) and AUCg (h^2 range from 0.45 to 0.52) of CAR. No significant heritabilities were found in AUCi of CAR and diurnal slope of cortisol level. For 24-hour urinary cortisol level, correlation could only be found in sibling pairsand hence no heritability was identified.

Table 46 Familial correlations of serial salivary cortisol profiles among family members

	Proband-Mother pairs	Proband-Father pairs	Proband-Sibling pairs	Father-mother pairs	Heritability ± SE
Salivary cortisol	N=164	N=143	N=85	N=113	
T1 corsitol	0 172*	0 160	0 130	0 310***	0 28±0 10**
T2 corsitol	0 317***	0 016	0 300*	0 035	0 39±0 11***
T3 corsitol	0 310***	0 020	0 319**	0 077	0 39±0 10***
T4 corsitol	0 213**	0 264**	0 071	0 217*	0 44±0 10***
T5 corsitol	0 189*	0 294***	0 274*	0 136	0 42±0 11***
T6 corsitol	0 258**	0 240**	0 107	0 102	0 45±0 10***
T7 corsitol	0 301***	0 285**	0 421***	0 139	0 45±0 10***
AUCg1	0 199*	0 034	0 232*	0 276*	0 45± 0 10***
AUCg2	0 253**	0 025	0 232*	0 255**	0 52±0 10***
AUCg3	0 276**	0 072	0 223**	0 261**	0 52±0 10***
AUCi1	0 019	0 022	0 168	0 225*	0 05±0 11
AUCi2	-0 003	0 012	0 200	0 227*	0 01±0 11
AUCi3	-0 028	0 011	0 214*	0 238**	0 00±0 50
Cortisol slope 1	0 093	-0 025	0 222*	0 205*	0 03±0 10
Cortisol slope 2	0 081	-0 039	0 124	0 112	0 16±0 10
Cortisol slope 3	0 157	0 046	0 304**	0 146	0 02±0 11
24-hour urinary cortisol	N=73	N=73	N=41	N=59	
#24-hour urinary cortisol	-0 119	0 052	0 322*	0 010	0 00±0 50

Spearman correlation analysis (correlation coefficients).

Heritability was adjusted for age and gender

*p<0.05; **p<0.01; ***p<0.001

3.3.6 Familial clustering in sleep quantity and sleep quality as measured by three-day actigraphy and questionnaire

Table 47 shows that more correlations among family members were found in subjective assessments of sleep parameters than in objective assessments. For actigraphic parameters, there were significant correlations between proband-mother pairs in TIB (r=0.20), sleep onset latency (r=0.231) and sleep efficiency (r=0.243). Correlation could only be found in sleep efficiency between proband-father pairs. There were significant correlations between sibling pairs in TIB (r=0.301), AST (r=0.218) and sleep onset latency (r=0.221).For subjective sleep parameters, there were significant correlations between family members on various sleep parameters except sleep onset latency.

Genetic analysis found that there were significant heritability in objective SOL (h^2±SE = 0.37±0.10, P<0.001) and sleep efficiency (h^2±SE = 0.29±0.12, P<0.05) and subjective TIB (h^2±SE = 0.35±0.10, P<0.001), sleep efficiency (h^2±SE = 0.46±0.10, p<0.001) and WASO (h^2±SE = 0.57±0.10, P<0.001).

Table 47 Familial correlations in objective and subjective sleep quality and quantity

	Proband-Mother pairs	Proband-Father pairs	Proband-Sibling pairs	Father-mother pairs&	Heritability ± SE
Actigraphy	N=136	N=125	N=96	N=97	
Time in bed, min	0.200*	0.093	0.301**	0.301**	0.17±0.13
Sleep onset latency, min	0.231*	0.000	0.221*	0.201	0.37±0.10***
Wake after sleep onset, min	0.049	-0.115	0.140	0.027	0.0±0.50
Sleep efficiency, %	0.243**	0.186*	0.073	-0.027	0.29±0.12*
Questionnaire	N=139	N=124	N=93	N=100	
Time in bed, min	0.212*	0.226*	0.379**	0.201	0.35±0.10***
Sleep onset latency, min	0.077	-0.013	0.057	-0.095	0.08±0.14
Sleep efficiency, %	0.385**	0.192*	0.248*	0.393***	0.46±0.10***
Wake after sleep onset, min	0.410**	0.314**	0.204	0.364**	0.57±0.10***

Heritability was adjusted for age and gender

Spearman correlation analysis (correlation coefficients).

*p<0.05; **p<0.01; ***p<0.001

3.3.7 Relationship between serial salivary cortisol level and 3-day objective sleep quantity and quality in adolescents

Tables 48 and 49 show the correlations between cortisol levels and sleep parameters as measured by three-day actigraphy and questionnaire in adolescents. There was a pattern that cortisol levels before (10:00 pm) and after (0 minute after waking up) bedtime were associated with poor subjective sleep quality (lower sleep efficiency and longer WASO) and quanlity. For example, cortisol levels at 10 pm were negatively correlated with subjective sleep efficiency ($r=-0.195$, $p<0.01$) and actual sleep time ($r=0.180$, $p<0.01$) but positively correlated with wake after sleep onset ($r=0.183$, $p<0.01$). Similar results were found in the relationship of cortisol level at 0 min after waking up. The results suggested that adolescents with higher cortisol levels before and after sleep reported a shorter sleep time and poor sleep quality.

In contrast, there was no correlation between objective sleep parameters and serial salivary cortisol except for negative correlation between TIB and diurnal slope 2 (at 4 pm time point).

Table 48 Correlation between sleep parameters and salivary cortisol levels in adolescents

	T1	T2	T3	T4	T5	T6	T7
Actigraphy (n=197)							
Time in bed, min	-0.096	-0.058	-0.025	0.083	0.048	0.125	0.035
Actual sleep time, min	-0.086	0.000	-0.023	0.036	0.061	0.140	0.025
Sleep onset latency, min	-0.028	-0.132	-0.019	0.072	-0.004	-0.019	0.011
Wake after sleep onset, min	-0.065	-0.019	0.033	0.075	0.036	0.001	-0.029
Sleep efficiency, %	-0.008	0.090	0.000	-0.059	-0.004	0.014	0.021
Questionnaire, n=212							
Time in bed, min	-0.197*	-0.033	0.035	0.037	0.013	0.100	-0.088
Actual sleep time, min	-0.229**	-0.059	0.024	0.023	-0.021	0.002	-0.180**
Sleep onset latency, min	0.041	0.068	0.130	0.041	0.112	0.222**	0.060
Sleep efficiency, %	-0.174*	-0.032	0.006	0.001	-0.049	-0.134	-0.195**
Wake after sleep onset, min	0.139*	0.030	-0.022	0.009	0.030	0.089	0.183**

Spearman correlation analysis. *p<0.05; **p<0.01; ***p<0.001

Table 49 Correlation between sleep parameters and salivary cortisol profile in adolescents

	AUCg1	AUCg2	AUCg3	AUCi1	AUCi2	ACUi3	Slope 2
Actigraphy (n=199)							
Time in bed, min	-0.085	-0.069	-0.041	0.039	0.050	0.075	-0.193**
Actual sleep time, min	-0.041	-0.029	-0.017	0.102	0.108	0.118	-0.007
Sleep onset latency, min	-0.096	-0.076	-0.049	-0.085	-0.052	0.002	0.029
Wake after sleep onset, min	-0.043	-0.031	-0.014	0.021	0.033	0.043	-0.101
Sleep efficiency, %	0.064	0.059	0.038	0.118	0.102	0.076	-0.116
Questionnaire, n=214							
Time in bed, min	-0.134	-0.115	-0.100	0.099	0.116	0.135*	-0.143*
Actual sleep time, min	-0.122	-0.109	-0.100	0.034	0.048	0.069	-0.065
Sleep onset latency, min	0.084	0.111	0.107	0.030	0.026	0.013	-0.084
Sleep efficiency, %	-0.067	-0.083	-0.091	-0.054	-0.070	-0.066	0.080
Wake after sleep onset, min	0.033	0.043	0.051	0.038	0.055	0.057	-0.025

Spearman correlation analysis. No significant correlations were found in slope 1 and slope 3, which were not presented.

*p<0.05; **p<0.01; ***p<0.001

3.3.8 Correlation between serial salivary cortisol and sleep parameters in adolescents using dichotomized classification

Tables 50-53 further delineate the relationship between cortisol levels and sleep parameters using dichotomized methods in adolescents. Objective short sleepers as defined as TIB $\leq$ 8 hours (accounted for around 30% of the samples) had higher diurnal slope 2 (4 pm) and slope 3 (10 pm) (0.68±0.28 vs 0.52±0.49 for slope 2 and 0.82±0.12 vs 0.74±0.25 for slope 3 respectively, $p<0.05$) than long/normal sleepers

(as defined as TIB > 8 hours). Adolescents with objective SE≥ 85% had higher cortisol increase than those adolescents with SE < 85% (P<0.05). Those adolescents with objective SOL >30 min had lower cortisol level at 30 min after waking up (8.6±4.4 vs 11.7±6.8, p=0.018) and lower AUCi1 (-1.4 ± 4.4 vs 1.8 ± 7.0, p=0.007) and AUCi2 (-3.0±6.4 vs 0.7±9.5, p=0.014) of CAR. Subjective short sleepers as defined by subjective TIB < 8 hours had higher cortisol levels at 0 min after waking up (11.7±5.7 nmol/L vs 9.5±4.5 nmol/L, p=0.009) , higher AUCg1 (23.8±8.9 nmol/L vs 20.9±0.6 nmol/L, p=0.022) and higher AUCg2 (28.8±14.1 nmol/L vs 31.5±11.5 nmol/L, p=0.046) than subjective long/normal sleepers. Subjective poor sleepers as defined by sleep efficiency < 85% had higher cortisol levels at 4 pm (6.9±6.3 nmol/L vs 3.4±2.6 nmol/L, p=0.006) and 10 pm (3.5±2.6 nmol/L vs 1.9±1.3 nmol/L, p=0.014).

Table 50 Comparison in cortisol levels between cortisol and objective sleep parameters in adolescents

	Time in bed > 8 hours (n=139)	Time in bed ≤ 8 hours (n=60)	P value	SE< 85% (n=131)	SE≥85% (n=68)	P value
T1, nmol/L	9 5±4 3	11 0±5 7	0 144	10 1±4 7	9 7±5 1	0 374
T2, nmol/L	11 0±7 0	11 6±5 7	0 317	10 6±6 1	12 4±7 3	0 102
T3, nmol/L	7 5±4 9	7 7±4 0	0 609	7 3±4 0	7 9±5 8	0 998
T4, nmol/L	5 9±4 1	5 4±3 6	0 648	5 7±3 7	5 9±4 5	0 849
T5, nmol/L	4 1±3 0	3 5±2 1	0 325	4 0±3 0	3 8±2 3	0 827
T6, nmol/L	3 8±3 3	2 8±2 0	0 052	3 5±3 1	3 6±2 7	0 624
T7, nmol/L	2 0±1 4	1 7±1 1	0 346	1 9±1 4	2 0±1 3	0 420
AUCg1, nmol/L	20 5±9 2	22 5±9 6	0 210	20 6±9 0	22 0±10 0	0 448
AUCg2, nmol/L	28 0±13 4	30 0±12 4	0 269	27 9±12 1	30 0±14 8	0 507
AUCg3, nmol/L	33 9±16 3	35 3±14 6	0 391	33 5±14 5	35 9±18 1	0 617
AUCi1, nmol/L	1 5±7 0	0 5±6 1	0 856	0 5±6 1	2 6±7 6	0 025*
AUCi2, nmol/L	0 6±9 2	-1 3±8 7	0 709	-0 9±8 2	1 8±10 4	0 047*
AUCi3, nmol/L	-2 2±13 5	-5 8±14 1	0 471	-4 5±12 5	0 9±15 7	0 075
Cortisol slope 1	0 42±0 75	0 57±0 37	0 071	0 47±0 73	0 45±0 51	0 807
Cortisol slope 2	0 52±0 49	0 68±0 28	0 005*	0 59±0 38	0 53±0 54	0 733
Cortisol slope 3	0 74±0 25	0 82±0 12	0 078	0 77±0 23	0 74±0 22	0 295

Non-parametric test * $p<0.05$

Table 51 Comparison between cortisol level and objective sleep onset latency in adolescents

	SOL ≤30 min N=163	SOL>30 min N=36	P value
T1, nmol/L	9.9±4.8	10.1±4.9	0.831
T2, nmol/L	11.7±6.8	8.6±4.4	0.018*
T3, nmol/L	7.7±4.7	6.8±4.2	0.353
T4, nmol/L	5.6±3.9	6.5±4.5	0.515
T5, nmol/L	3.9±2.6	4.1±3.4	0.417
T6, nmol/L	3.5±2.6	3.5±4.2	0.120
T7, nmol/L	1.9±1.3	2.0±1.4	0.765
AUCg1, nmol/L	21.6±9.6	18.9±8.2	0.092
AUCg2, nmol/L	29.2±13.3	25.7±11.6	0.143
AUCg3, nmol/L	34.8±15.9	32.1±15.1	0.322
AUCi1, nmol/L	1.8±7.0	-1.4±4.4	0.007*
AUCi2, nmol/L	0.7±9.5	-3.0±6.4	0.014*
AUCi3, nmol/L	-2.6±14.2	-6.4±11.3	0.100
Cortisol slope 1	0.45±0.69	0.50±0.49	0.778
Cortisol slope 2	0.59±0.54	0.56±0.42	0.194
Cortisol slope 3	0.76±0.23	0.76±0.22	0.994

Non-parametric test * $p<0.05$

Table 52 Comparison between cortisol level and subjective sleep parameters in adolescents

	Time in bed > 8 hours (n=149)	Time in bed ≤ 8 hours (n=65)	P value	SE< 85% (n=11)	SE≥85% (n=198)	P value
T1, nmol/L	9 5±4 5	11 7±5 7	0 009*	11 7±7 6	9 9±4 8	0 735
T2, nmol/L	11 3±7 2	12 1±5 5	0 108	11 5±8 5	11 6±6 7	0 639
T3, nmol/L	7 9±5 3	7 7±3 8	0 359	7 4±4 4	7 9±5 0	0 608
T4, nmol/L	5 8±4 0	5 9±3 7	0 388	6 1±4 5	5 8±3 9	0 955
T5, nmol/L	4 0±2 8	4 2±2 6	0 526	5 4±3 3	4 0±2 7	0 089
T6, nmol/L	3 7±3 2	3 3±2 4	0 679	6 9±6 3	3 4±2 6	0 006*
T7, nmol/L	1 9±1 4	2 1±1 5	0 446	3 5±2 6	1 9±1 3	0 014*
AUCg1, nmol/L	20 9±6 6	23 8±8 9	0 022*	23 3±10 8	21 5±9 5	0 703
AUCg2, nmol/L	28 8±14 1	31 5±11 5	0 046*	30 6±13 9	29 4±13 5	0 852
AUCg3, nmol/L	34 5±16 9	37 5±13 9	0 058	36 7±16 1	35 3±16 3	0 834
AUCi1, nmol/L	1 8±7 3	0 4±6 8	0 453	-0 2±11 9	1 6±6 8	0 527
AUCi2, nmol/L	1 0±9 9	-1 6±9 5	0 243	-2 4±15 8	0 6±9 3	0 634
AUCi3, nmol/L	-1 7±14 6	-6 5±14 9	0 150	-7 4±23 7	-2 4±14 0	0 745
Cortisol slope 1	0 49±0 41	0 53±0 49	0 128	0 34±0 65	0 51±0 42	0 329
Cortisol slope 2	0 55±0 44	0 67±0 30	0 018*	0 32±0 39	0 60±0 41	0 010*
Cortisol slope 3	0 76±0 19	0 80±0 16	0 227	0 66±0 21	0 78±0 18	0 026*

Non-parametric test * $p<0.05$

Table 53 Comparison between cortisol level and subjective sleep onset latency in adolescents

	SOL ≤30 min N=189	SOL>30 min N=25	P value
T1, nmol/L	10 1±5 0	10 9±4 8	0 372
T2, nmol/L	11 5±6 8	12 3±6 4	0 221
T3, nmol/L	7 7±4 9	9 6±4 3	0 012*
T4, nmol/L	5 7±3 8	7 4±4 3	0 016*
T5, nmol/L	4 0±2 8	4 1±2 2	0 619
T6, nmol/L	3 4±2 9	4 2±3 1	0 175
T7, nmol/L	1 9±1 4	2 1±1 3	0 322
AUCg1, nmol/L	21 5±9 6	23 2±7 9	0 171
AUCg2, nmol/L	29 2±13 5	32 8±11 4	0 048*
AUCg3, nmol/L	34 9±16 3	40 2±14 0	0 031*
AUCi1, nmol/L	1 4±7 0	1 3±8 1	0 676
AUCi2, nmol/L	0 24±9 63	0 66±10 8	0 623
AUCi3, nmol/L	-0 31±14 6	-1 8±15 8	0 606
Cortisol slope 1	0 50±0 44	0 56±0 27	0 790
Cortisol slope 2	0 60±0 37	0 56±0 36	0 696
Cortisol slope 3	0 78±0 17	0 79±0 14	0 974

Non-parametric test * $p<0.05$

3.3.9 Correlation between serial salivary cortisol and sleep parameters in adults

Tables 54 shows that there was mild but significant negative correlation between subjective TIB and salivary cortisol level at 0 min after waking up ($r= -0.141$, $p<0.05$) in adults. The three AUCg of CAR were negatively correlated with subjective TIB (r ranging from -0.165 to -0.186, $p<0.05$) (table 55) while the three AUCi were positively correlated with subjective SOL (r ranging from 0.152 to 0.183, $p<0.05$).

3.3.10 Correlation between serial salivary cortisol and sleep parameters in adults using dichotomized classification

Tables 56-59 further delineate the relationship between cortisol levels and sleep parameters using dichotomized methods in adults. Objective short sleepers (as defined by objective TIB $\leq$ 400 minutes) had higher cortisol level at 30 min after waking up (14.0±6.0 nmol/L vs 11.5±6.2 nmol/L, $p=0.010$) and $AUCg_I$ (27.5±11.6 nmol/L vs 22.7±10.0 nmol/L, $p=0.018$). No differences were found between objective poor sleepers (objective SE < 85%) and objective good sleepers (objective SE $\geq$ 85%). Those adults with objective SOL > 30 min had less cortisol slope at 4 pm (0.49±0.31 nmol/L vs 0.63±0.31 nmol/L, $p=0.011$) than those with objective SOL $\leq$30 min. No

differences were found in salivary cortisol level between subjective short sleepers (subjective TIB $\leq$ 400 minutes) and subjective normal/long sleepers (subjective TIB > 400 minutes) and between subjective poor sleepers (subjective SE <85%) and subjective good sleepers (subjective SE $\geq$ 85%). Those adults with subjective SOL > 30 min had lower cortisol level at 0 min after waking up (9.7±4.2 nmol/L vs 12.3±5.9 nmol/L, p=0.017) but similar cortisol level at 30, 60 and 90 min with those with subjective SOL $\leq$ 30 min and hence had higher AUCi1, AUCi2 and AUCi 3 ($p<0.05$) than their counterparts.

Table 54 Correlation between sleep parameters and salivary cortisol levels in adults

	T1	T2	T3	T4	T5	T6	T7
Actigraphy (n=202)							
Time in bed, min	-0 113	-0 107	0 078	0 020	0 104	0 000	0 094
Actual sleep time, min	-0 085	-0 061	0 050	-0 012	0 020	-0 085	0 032
Sleep onset latency, min	-0 101	-0 060	-0 011	-0 033	0 105	0 126	0 064
Wake after sleep onset, min	0 074	0 116	0 018	0 008	0 049	-0 033	0 049
Sleep efficiency, %	0 049	0 098	-0 048	-0 068	-0 089	-0 028	-0 072
Questionnaire, n=221							
Time in bed, min	-0 141*	-0 117	-0 036	-0 095	0 052	0 038	0 034
Actual sleep time, min	-0 054	-0 108	-0 072	-0 086	0 039	-0 024	0 018
Sleep onset latency, min	-0 081	0 102	0 135*	0 102	0 031	0 127	0 005
Sleep efficiency, %	0 107	0 039	-0 021	0 040	0 044	-0 036	0 007
Wake after sleep onset, min	-0 089	-0 062	-0 010	-0 065	-0 013	0 028	0 004

Spearman correlation analysis

*p<0 05, **p<0 01, ***p<0 001

Table 55 Correlation between sleep parameters and salivary cortisol profile in adults

	AUCg1	$AUCg_2$	$AUCg_3$	AUCi1	AUCi2	ACUi3	Slope 2	Slope 3
Actigraphy (n=195)								
Time in bed, min	-0.121	-0.096	-0.094	-0.028	0.021	0.060	-0.082	-0.096
Actual sleep time, min	-0.110	-0.092	-0.093	-0.006	0.029	0.053	-0.022	-0.069
Sleep onset latency, min	-0.043	-0.052	-0.052	0.026	0.026	0.029	-0.152*	-0.065
Wake after sleep onset, min	0.060	0.045	0.034	0.011	0.003	0.006	-0.019	-0.026
Sleep efficiency, %	-0.003	0.003	-0.002	0.082	0.063	0.044	0.102	0.042
Questionnaire, n=208								
Time in bed, min	-0.186**	-0.167*	-0.165*	-0.021	0.002	0.031	-0.112	-0.146*
Actual sleep time, min	-0.156*	-0.170*	-0.172*	-0.086	-0.090	-0.077	0.000	-0.083
Sleep onset latency, min	-0.024	-0.002	-0.007	0.152*	0.172*	0.183**	-0.138*	-0.009
Sleep efficiency, %	0.058	0.040	0.044	-0.037	-0.074	-0.097	0.081	0.052
Wake after sleep onset, min	-0.055	-0.044	-0.043	-0.022	0.008	0.029	-0.036	-0.069

Spearman correlation analysis.

*p<0.05; **p<0.01; ***p<0.001

Data for cortisol slope 1 was not presented as there was not statistically significant.

Table 56 Comparison between cortisol level and objective sleep parameters in adults

	TIB > 400 min (n=154)	TIB ≤ 400 minutes (n=38)	P value	SE< 85% (n=99)	SE≥85% (n=93)	P value
T1, nmol/L	11 2±5 0	13 5±7 9	0 243*	12 1±6 4	11 0±5 0	0 265
T2, nmol/L	11 5±6 2	14 0±6 0	0 010*	12 2±6 4	11 7±5 9	0 856
T3, nmol/L	8 3±4 7	8 2±4 2	0 625	8 3±4 8	8 0±4 4	0 806
T4, nmol/L	6 2±3 7	6 7±3 8	0 236	6 5±3 7	6 0±3 7	0 416
T5, nmol/L	4 8±3 3	4 6±2 3	0 466	5 1±3 5	4 4±2 6	0 114
T6, nmol/L	3 9±2 6	4 5±4 1	0 416	4 3±2 9	3 8±3 1	0 077
T7, nmol/L	2 2±1 8	2 1±1 6	0 831	2 3±1 8	2 1±1 7	0 428
AUCg1, nmol/L	22 7±10 0	27 5±11 6	0 018*	24 6±11 3	22 7±9 6	0 407
AUCg2, nmol/L	30 9±14 2	35 8±14 6	0 047*	33 0±15 2	30 8±13 4	0 549
AUCg3, nmol/L	37 0±17 4	42 6±17 2	0 044*	39 5±18 5	36 7±16 3	0 518
AUCi1, nmol/L	0 2±5 3	0 4±7 8	0 384	0 7±5 4	-0 2±6 2	0 139
AUCi2, nmol/L	-1 3±7 2	-2 2±10 7	0 934	-2 2±8 5	-0 7±7 3	0 123
AUCi3, nmol/L	-5 4±10 9	-8 2±16 7	0 631	-7 2±13 1	-4 7±11 3	0 133
Cortisol slope 1	0 50±0 51	0 55±0 33	0 481	0 47±0 59	0 54±0 33	0 487
Cortisol slope 2	0 62±0 28	0 58±0 44	0 667	0 60±0 32	0 62±0 31	0 349
Cortisol slope 3	0 77±0 30	0 78±0 21	0 424	0 78±0 18	0 76±0 37	0 947

Non-parametric test * $p<0.05$

TIB time in bed, SE sleep efficienty

Table 57 Comparison between cortisol level and objective sleep parameters in adults

	SOL ≤30 min N=171	SOL>30 min N=21	P value*
T1, nmol/L	11 9±5 7	9 9±4 3	0 507
T2, nmol/L	12 4±6 2	10 9±5 5	0 953
T3, nmol/L	8 3±4 6	8 3±4 4	0 603
T4, nmol/L	6 4±3 8	5 9±3 1	0 625
T5, nmol/L	4 7±2 8	5 9±3 9	0 268
T6, nmol/L	3 8±2 9	5 5±3 2	0 108
T7, nmol/L	2 2±1 7	2 4±2 1	0 835
AUCg1, nmol/L	23 8±10 7	22 7±9 1	0 828
AUCg2, nmol/L	32 0±14 6	31 0±12 6	0 899
AUCg3, nmol/L	38 2±17 7	37 2±15 1	0 977
AUCi1, nmol/L	0 2±5 9	1 1±5 0	0 597
AUCi2, nmol/L	-1 7±8 2	-0 2±6 1	0 543
AUCi3, nmol/L	-6 2±12 7	-3 7±8 5	0 516
Cortisol slope 1	0 54±0 31	0 20±1 11	0 135
Cortisol slope 2	0 63±0 31	0 49±0 31	0 019*
Cortisol slope 3	0 77±0 30	0 78±0 15	0 514

Non-parametric test * $p<0.05$

SOL: sleep onset latency

* $p<0.05$

Table 58 Comparison between cortisol level and subjective sleep parameters in adults

	TIB > 400 min (n=168)	TIB ≤ 400 min (n=46)	P value	SE< 85% (n=33)	SE≥85% (n=172)	P value
T1, nmol/L	11.4±5.1	13.1±7.3	0.308	11.0±4.3	11.9±6.0	0.682
T2, nmol/L	12.3±6.7	14.0±6.4	0.050	13.0±5.8	12.5±6.7	0.347
T3, nmol/L	8.6±5.1	9.3±4.3	0.188	9.5±4.6	8.5±4.9	0.189
T4, nmol/L	6.3±3.8	7.4±4.6	0.156	6.6±3.0	6.5±4.1	0.365
T5, nmol/L	4.8±3.1	5.0±2.5	0.294	5.1±3.8	4.8±2.8	0.786
T6, nmol/L	4.1±2.9	4.2±2.9	0.563	4.6±3.3	4.0±2.7	0.300
T7, nmol/L	2.3±1.7	2.7±2.1	0.500	2.2±1.8	2.5±1.9	0.353
AUCg1, nmol/L	23.7±10.6	27.1±11.6	0.084	24.1±8.6	24.4±11.2	0.613
AUCg2, nmol/L	32.2±15.1	36.4±15.1	0.097	33.5±12.1	32.9±15.6	0.390
AUCg3, nmol/L	38.6±18.4	43.8±18.6	0.082	40.1±14.7	39.4±19.1	0.388
AUCi1, nmol/L	0.8±5.4	1.0±7.4	0.285	2.1±5.4	0.6±5.9	0.139
AUCi2, nmol/L	-0.5±7.1	-0.9±10.3	0.468	1.3±7.2	-1.1±7.9	0.065
AUCi3, nmol/L	-4.5±10.5	-5.7±16.1	0.722	-1.7±10.7	-5.6±12.0	0.055
Cortisol slope 1	0.50±0.50	0.53±0.32	0.926	0.34±0.94	0.54±0.30	0.711
Cortisol slope 2	0.61±0.29	0.62±0.34	0.683	0.57±0.25	0.61±0.30	0.132
Cortisol slope 3	0.77±0.29	0.74±0.22	0.957	0.80±0.14	0.75±0.30	0.559

Non-parametric test * $p<0.05$

TIB: time in bed; SE: sleep efficienty

* $p<0.05$

Table 59 Comparison between cortisol level and subjective sleep parameters in adults

	SOL ≤30 min N=172	SOL>30 min N=36	P value
T1, nmol/L	12.3±5.9	9.7±4.2	0.017*
T2, nmol/L	12.6±6.7	13.0±6.8	0.721
T3, nmol/L	8.7±4.9	8.7±5.1	0.958
T4, nmol/L	6.6±4.0	6.2±3.8	0.722
T5, nmol/L	4.9±2.9	5.0±3.5	0.725
T6, nmol/L	4.0±2.7	4.8±3.7	0.295
T7, nmol/L	2.5±1.9	2.1±1.8	0.133
AUCg1, nmol/L	24.9±11.1	22.7±10.1	0.358
AUCg2, nmol/L	33.6±15.2	31.4±14.8	0.516
AUCg3, nmol/L	40.2±18.6	37.7±18.2	0.513
AUCi1, nmol/L	0.4±6.0	3.3±5.1	0.003*
AUCi2, nmol/L	-1.4±8.1	2.8±6.2	0.002*
AUCi3, nmol/L	-6.0±12.3	0.6±8.3	0.001*
Cortisol slope 1	0.52±0.48	0.45±0.39	0.191
Cortisol slope 2	0.63±0.28	0.49±0.34	0.008*
Cortisol slope 3	0.76±0.29	0.76±0.27	0.607

Non-parametric test * $p<0.05$

SOL: sleep onset latency

* $p<0.05$

3.3.11 Correlation between twenty-four hour urinary cortisol and sleep parameters

Table 60 shows the correlations between 24-hour cortisol level and sleep parameters in both adolescents and adults. Interestingly, significant correlations could only be found in adolescents. 24-hour urinary cortisol level was negatively correlated with objective and subjective TIB ($r = -0.373$ for objective measure and $r=-0.267$ for subjective measure respectively, $p<0.01$), AST ($r = -0.372$ for objective measure and $r= -0.386$ for subjective measure respectively, $p<0.001$) and subjective sleep efficiency ($r = -0.315$, $p<0.001$). 24-hour urinary cortisol level was positively correlated with subjective WASO ($r=0.235$, $p<0.025$).

Table 60 Correlations between 24-hour urinary cortisol level and sleep parameters in adults and adolescents

	Adolesecents	Adults
Actigraphy	N=84	N=96
Time in bed, min	-0.373***	-0.004
Actual sleep time, min	-0.372***	0.124
Sleep onset latency, min	-0.014	-0.143
Wake after sleep onset, min	-0.241	0.041
Sleep efficiency, %	-0.032	-0.117
Questionnaire	N=103	N=112
Time in bed, min	-0.267**	-0.047
Actual sleep time, min	-0.386***	-0.002
Sleep onset latency, min	-0.118	-0.032
Sleep efficiency, %	-0.315***	0.078
Wake after sleep onset, min	0.253*	-0.021

Spearman correlation analysis

*Adjusted significant P value was < 0.025 (adjusted for multiple comparisons).

*p<0.025; **p<0.01; ***p<0.001.

DISCUSSION

CHAPTER FOUR

LONG-TERM COURSES AND OUTCOMES OF INSOMNIA

4.1 Longitudinal courses of insomnia in children (adolescents)

Although insomnia has been generally suggested as chronic problem in adults, only limited information is known about the long-term course of insomnia in children and adolescents. Our study found that although insomnia was commonly found at both baseline (4.2%) and 5-year follow-up (6.6%), the persistence rate of insomnia symptoms was only 14.9% (or 27.4% with loose criteria), which was slightly lower than other previous studies (21-60%).[24, 29, 124] The differences between ours and other studies might be due to several differences in the study designs. The longitudinal course in our study with a period of 4.7 years of follow-up was longer than previous studies which had only one to two years duration. The second reason might be the relatively strict criteria that is employed in our study to define insomnia with a frequency of $\geq$3 times /week over a duration of past 12-month. Other studies also suggested that by using stricter criteria of insomnia (plus daytime fatigue and/or sleepiness), the persistence rate of insomnia would decrease from 45.8% to 22.8%[29]

and from 52% to 21% respectively.[124] Consistent with these studies, the persistence and incidence rates of our study increased when we used loose criteria (≥ 1 times/week) in both children and adults.

Our further analysis in logistics regression showed that several factors were associated with incidence of insomnia in adolescents, including lower paternal education level (AOR =2.49), frequent temper outbursts (AOR =1.85) and feeling tired at daytime (AOR =2.17), while persistence of insomnia in this group was associated with their chronic medical condition(s) (AOR =10.2) and nocturnal sweating (AOR =5.15). Although previous study in adolescents found that there was no major association between socio-demography and incidence or persistence of insomnia, our data suggested that new onset of insomnia may be related to sociodemographics in the family and daytime functional impairments (fatigue and temper outbursts) of adolescents. On the other hand, presence of chronic medical conditions and nocturnal sweating might predict the persistence of insomnia in children. The high remission rate of insomnia from children to adolescents suggested that insomnia has waxed and waning course. As there was relative small sample size in insomniac children at baseline, further studies are warranted to investigate the factors associated with course of insomnia in children.

4.2 Longitudinal course of insomnia in middle-aged adults

Our study found similar patterns of long-term course of insomnia in adults with incidence rates of 5.4% and 6.8% and persistence rate of 26.9% and 43.8% for males and females respectively. Female predominance in prevalence of insomnia was found at both baseline and follow-up which is similar to previous epidemiologic and meta-analysis studies.[20, 23] Female predominance of insomnia has age-dependent effect with being more prominent in elderly than young adults.[20] Our study found that the gender differences in insomnia could only be found in middle-aged adult but not adolescents. Thus, our longitudinal study suggested that the higher prevalence of insomnia in female adults might be due to its higher persistence rate rather than higher incidence rate. The mechanism underlying gender differences of insomnia was unclear. A recent study suggested that gender differences in insomnia became statistically insignificant after adjusting for mental disorders.[125] This study proposed that the psychiatric comorbidity might be a mediator between gender and insomnia.[125] However, our study showed that the gender difference in prevalence and persistence of insomnia at follow-up persisted even after controlling for current psychiatric disorders. In addition, there was no association between gender and incidence of insomnia in both univariate analysis and logistic regression. The results of our study

suggested that psychiatric disorders could not completely explain the gender differences in both persistence and prevalence of insomnia. Further longitudinal studies with parallel assessments on both psychiatric disorders and insomnia are needed to find out the definitive role of psychiatric disorders on gender differences of insomnia.

On the other hand, little is known about the gender differences in insomnia among adolescents. A few studies found that female predominance could also be found in adolescents, [28, 29, 42] while others did not find gender differences just like our study.[46] In a study which found gender differences in insomnia, it suggested that menstruation onset might account for the gender difference of insomnia.[28] However our study neither found any differences in the rate of insomnia across different pubertal statuses nor found any gender differences in rate of insomnia in adolescent probands and their siblings. Hence, the gender differences in adolescent insomnia, their exact timing and potential mechanism need further investigations.

4.3 Medical consequences of insomnia at baseline in adolescents and adults

Previous cross-sectional studies suggested that insomnia was a common symptom of

other physical diseases which might be related to the distress or medical consequences as caused by these conditions.[126-129] Thus, it was used to be called insomnia due to medical condition or comorbid insomnia.[130] Our studies found that insomnia at baseline was strongly associated with several upper airway inflammatory diseases, such as allergic rhinitis, asthma and laryngopharygngitis in both children and adults after 5 years of follow-up. In other words, our findings suggested that chronic insomnia is a predisposing factor of these diseases. The mechanism underlying insomnia and these diseases is unclear. It seems not to be related to lower socio-demographic background in insomniacs as most of the associations persisted even after controlling for age, gender, parental education and family income. As all these diseases were related to inflammation or dysregulation of immune functioning, the decreased immune functioning as related to chronic insomnia might account for this phenomenon. Previous study found that chronic insomniacs had lower levels of CD3+, CD4+ and CD8+ cells than good sleepers.[131] Another study found that insomniacs had lower natural killer cell responses.[132] These studies suggested that the immune alterations might play a critical role in the relationship between insomnia and these upper airway inflammatory diseases. In his review, Irwin suggested that there was a reciprocal interaction between sleep and cytokines and immune system based on previous experimental studies.[132] An experimental study found that those subjects

with poorer sleep efficiency over the past 14 days were 5.5 times more likely to develop a cold than good sleepers when they received a nasal drop containing rhinovirus.[133] Our study further shows that chronic insomnia at baseline could strongly predict several upper airway inflammatory diseases which are associated with dysregulations of immune functioning in general population. The consistency of the association in both children and adults suggested that these findings are pertinent. However, due to relatively small sample size of these diseases in those subjects with insomnia, we could not further analyze the effects of remission and persistence of insomnia on these diseases. Further studies are needed to examine the effects of treatment of insomnia on the prevention of these immune-related inflammatory diseases.

4.4 Insomnia and chronic medical conditions at follow-up in adults

4.4.1 Insomnia and hypertension

The relationship between insomnia and hypertension was controversial in previous studies. Longitudinal study in Japanese male workers found that persistent insomnia predicted hypertension[6] while another cohort could not find any association in older

adults[7, 8] or even reverse association between insomnia and incident hypertension was found in subgroup analysis.[7] More recently, the First National Health and Nutrition Examination Survey (NHANES I) found that both insomnia and short sleep duration were associated with incidence of hypertension.[134] Our study employing self-reported hypertension found that insomniacs at baseline were 1.73 times at risk to develop hypertension after 5 years. One should be noted that three studies with positive findings were conducted on middle-aged adults while the negative one was conducted on elderly. The age differences among these studies might account for the inconsistency as subgroup analysis in NHANES I further revealed that there was no significant association between insomnia and hypertension in elderly. Previous studies also found differential age effects of other sleep disturbances on cardiovascular and metabolic systems. The association between short sleep duration and hypertension was strongest in premenopausal women,[135] while the association between short sleep duration and obesity was stronger in children than adults by a recent meta-analysis. [136] Further studies on different age and gender groups are needed to delineate the relationship between insomnia and hypertension.

The mechanism underlying the relationship between insomnia and hypertension might be mediated through several pathways. Chronic sleep loss induced by persistent

insomnia might be involved into this relationship as short sleep duration has been found to correlate with both obesity[136] and hypertension.[137] Insomnia and short sleep duration has been found to have synergistic effects on hypertension.[5] Second, insomnia is highly comorbid with depression[10] while depression has been found to be associated with hypertension in both cross-sectional studies[138, 139] and longitudinal studies. [134, 140, 141] The relationship between depression and hypertension has been found to slightly attenuate after controlling for insomnia and sleep duration in NHANES I study. This study suggested that insomnia and sleep duration might serve as mediators in the relationship between depression and hypertension, but it did not test whether depression could serve as a mediator in the relationship between hypertension and insomnia. Further studies should also pay attention to this issue as insomnia was more likely to be a precursor or comorbid condition of depression.[10] Third, the association between insomnia and hypertension might be mediated by increased stress related hormones as related to insomnia or sleep loss. Our previous study found that poor sleep quality as measured by actigraphy was associated with increased 24-hour urinary catecholamines (adrenaline and noradrenaline) in normal subjects. Our phase 3 study also found that short sleep duration and poor sleep quality as reported by questionnaire were correlated with increased 24-h urinary cortisol, especially for adolescents (further discussed below).

4.4.2 Insomnia, chronic pain and arthritis

Insomnia is a common symptom in chronic pain and arthritis.[142, 143] The treatment of insomnia could benefit not only insomnia but also painful symptoms in those subjects with chronic pain and insomnia.[144-146] Evidence from previous studies suggested that insomnia and chronic pain had interactive relationship.[147] However, the relationship between insomnia and chronic pain was not consistent in longitudinal studies[147] and most of which only focused on the impact of chronic pain on insomnia with short follow-up period.[148-151] Our study extend previous findings that insomnia was also an independent risk factor for chronic pain and arthritis that were severe enough to require clinical treatments. The mechanism underlying chronic pain and insomnia might be mediated by a decrease of sleep wave sleep (SWS) [101] as decreased SWS sleep was correlated with pain severity in patients with chronic pain and rheumatic diseases.[152-154] Our phase 2 study further supported the close quantitative association between insomnia and pain problems. In addition, comorbidity between insomnia and pain were accounted by both genetic and environmental factors (further discussed below).

4.4.3 Insomnia and psychiatric disorders

The relationship between insomnia and psychiatric disorders has been well established by previous studies.[10] Our study found that not only insomnia but also subthreshold insomnia with a frequency of 1-2 times /week at baseline could independently predict psychiatric disorders after 5 years in adults. The mechanism underlying the relationship between insomnia and psychiatric disorders will be further discussed in the next chapter.

4.4.4 Insomnia and GERD

Increasing attention has been paid to the relationship between insomnia and gastroesophageal reflux disease (GERD).[155] Three recent studies supported the relationship between insomnia and GERD. Jansson et al found that subjects with insomnia were 3 times more likely to have GERD in a population-based, cross-sectional study with large sample size (n=65333).[156] The authors concluded that the relationship between insomnia and GERD was bidirectional. The second one found that individuals with GERD had two times at risk to have sleep difficulty than those

without GERD symptoms.[157] This study further demonstrated that subjects with insomnia and GERD had increased number of general physician visits and more work impairments as well as lower quality of life than those only with GERD.[157] The last one was conducted in Chinese population which found similar results with the previous studies that reflux symptoms were correlated with poor sleep quality with OR of 2.25.[158] Collectively, these previous studies found a positive correlation between GERD and insomnia/poor sleep. However, the nature of these studies with cross-sectional designs could not disentangle the causal relationship between insomnia and GERD. Furthermore, most of the studies only focused on the impact of GERD symptoms on sleep. One placebo-control trial found that zolpidem, a GABA A receptor antagonist, would reduce the arousal response but prolong the duration of esophageal reflux event in patients with GERD.[159] In his editorial, Harding proposed that GERD was a cause of insomnia and suggested that use of hypnotics should be cautioned in these patients as based on the prolongation of esophageal reflux as related to usage of hynotics.[155] However, our longitudinal study found that insomnia at baseline was an independent predictor of GERD which implied that the management of insomnia symptoms should benefit not only insomnia but also GERD. Nonetheless, a risk-benefit analysis of hypnotic usage is advised in treating comorbid insomnia with GERD.

4.5 Strengths and limitations of phase one

The merits of this study relied on the longitudinal design in a large community-based cohort. Second, there were only slight differences in socio-demographics between those recruited subjects and the dropped outs, which suggested little potential bias in this study. To increase the response rate, several strategies were employed to increase the response rate, such as remind phone calls and letters and repeated sending of questionnaires.

There are several limitations in phase 1. The current study was based on self-reported data without further assessment by structured interview and clinical examination. Our phase 2 study found that the self-reported insomnia had high specificity but rather low sensitivity with clinical interview in both adolescents and adults. Another limitation of this study is that the ascertainment of the medical conditions was only based on self-reported data without confirmation by other methods. In view of potential reporting bias for the chronic medical conditions, we emphasized on those medical conditions which had been diagnosed by physician and/or needed drug treatment to increase the specificity. Those subjects with these medical conditions but did not receive diagnosis

or treatment might be misclassified in our study. Finally, in a recent study with three annually assessment, insomnia has been found to have a relatively high remittion rate (around 40% in a period of 1-year) and a high relapse rate (around 30%).[35] Our study might miss those insomniacs with waxed and waning course within the 5-year period. The absence of assessment on lifetime insomnia at both baseline and follow-up could not allow us to explore the precise relapse of insomnia by a two-wave study.

4.6 Conclusions in phase one

Insomnia is commonly found in both adolescents and adults with moderate persistence rate after 5 years in Hong Kong Chinese. Our findings of increased risk of chronic medical burdens in subjects with insomnia suggested that insomnia is a disorder that needs comprehensive medical attention on both mental and physical aspects.

CHAPTER FIVE

FAMILIAL AGGREGATION AND HERITABILITY OF INSOMNIA: A CASE-CONTROL STUDY

5.1 Familial aggregation and heritability in insomnia

Previous studies including our own suggested that insomnia is a disorder with strong familial clustering phenomenon. [46-52] Our current study confirmed strong familial aggregation of insomnia with a first degree relatives' recurrence risk of 2.33 for current insomnia and 2.82 for lifetime insomnia respectively. Further genetic analysis found that insomnia is a highly heritable disorder with a heritability of 0.57 for current insomnia and 0.67 for lifetime insomnia, which is comparable to those findings of twin studies which showed that the heritability was ranged from 0.42 to 0.57 for sleep problems or poor sleep. [160-163] The strong heritability of a clinical diagnosis of insomnia was further in concordance with the significant heritability of various sleep parameters (h^2 = 0.29-0.57) in our phase 3 study (page 137). Our data was also comparable to another longitudinal study which suggested that the genetic factors accounted for 46% variance of stability of sleep problems after 2 years of

follow-up.[160] On the other hand, the heritability for insomnia severity (as measured by ISI, h^2 =0.27) and sleep quality (as measured by PSQI, h^2 =0.30) were slightly lower than insomnia disorder as determined by clinical interview in our study. These results suggested that the severity of insomnia or sleep quality may be contributed by stronger environmental component when compared with insomnia diagnosis. In addition, our study found that heritability of primary insomnia (both current and lifetime) still persisted even after excluding those subjects with psychiatric disorders but with slight decrease in strength.

Our findings that FIRST score could not be influenced by genetic factors were unexpected as previous study which suggested that 37.2% of variance of FIRST score could be accounted by familial aggregation in sibling pairs.[164] In fact, we could not find any significant correlation between family members in FIRST scores. A possible explanation for this discrepancy could be age differences between these two studies. The participants in previous one were relatively older with mean age of 51.1 ±12.1 years old. The genetic influence on other sleep aspects, such as sleep duration, could only be found in those subjects aged over 25 years but not younger subjects.[161] On the other hand, it is also possible that the genetic component plays less prominent role for the vulnerability to acute situation-related sleep disturbance (as indicated by FIRST).

5.2 Evidence of gene and environment interaction in the causation of insomnia

Increasing efforts have been put in investigating the gene-environment interactions of complex diseases in genetic epidemiology in recent three decades. There has been much discussion and arguments on how to define and measure interaction in this field.[165-167] To the best of our knowledge, this is the first study in the insomnia literature showing a gene-environment synergistic interaction on the pathogenesis of insomnia. Insomnia is a highly heritable disorder as shown in our data. Hence, we defined parental history of insomnia (lifetime) as a genetic liability and defined high life stress as environment factors. Adapting the statistical method as suggested by Darroch,[168] we showed that the parental history and life stress interaction on insomnia only occur in those families with both parents having histories of insomnia, which indicated the gene-environment interaction might probably only occur in those with more genetic load. The effect size (2.18 times) for gene-environment interaction was modest and close to the first degree recurrence risk as found in this study but was larger than the effect size of putative gene (5-HTTLPR) on primary insomnia (OR=1.34).[58] We found that in the group with highest risk (with high life stress and both parents with history of insomnia), over 70% could be identified to have clinical insomnia as asscertained by clinical interview.

Given the limited understanding of familial aggregation of insomnia, our current findings of strong familial aggregation, high heritability and significant gene-environment interaction in insomnia among community subjects are pertinent. The findings in this study could pave the way for the further delineation of the molecular genetics and its interaction with environment factors in insomnia.

The gene-environment interaction approach has been widely used in psychiatric disorders, which was first systematically proposed by Kendler and Eaves.[169] Several issues should be cautioned when interpreting our results. First, our study did not test any putative gene loci as a genetic risk. We employed parental history of insomnia as genetic proxy for the insomnia of their offspring in this study. The parental history of insomnia might be attributed to other environmental factors, although the recruitment of family history as genetic liability was a common practice in genetic epidemiology. Nonetheless, our genetic analysis by SOLAR analysis showed that insomnia had very high heritability with 61% of the variance of lifetime insomnia diagnosis being accounted by genetic component. Hence, the parental history of insomnia could be considered as a reliable genetic proxy in genetic analysis. Second, the assessment of both current insomnia and life event were based on the past 12-month prior to clinical

interview, the real-time relationship between reported life stress and insomnia in this study is unclear. Further studies with more refined prospective design should be needed to confirm our conclusion. Third, although our study suggested gene-environment interaction on pathogenesis of insomnia, the implications of these findings on clinical practice will need further investigations. In this regard, the molecular genetic control of insomnia should be further investigated on a variety of suspected genes, including 5-HTTLPR as found in previous study.[58] Finally, just like other studies testing gene-environment interaction effects, the environmental exposures are difficult to define and measure precisely. The putative environmental risk factors in our study might not be only purely environmental, which might also be influenced by genetic factors; which is known as "gene-environment correlation".[170, 171]

In addition, our study showed that there was stronger mother-offspring association in insomnia diagnosis than father-offspring association in phase 2 study, which was consistent with the findings in our baseline study and phase 1 study. The exact mechanism underlying this phenomenon is unclear. In our previous study, we proposed several poetential explanations for stronger mother-offspring association, such as more contact time between mother and offspring and genetic component via maternal transmission.[27] In Hong Kong Chinese, near half of mothers with a child

were housewifes or unemployed (table 5). The mothers might exert more impact on lifestyle establishments and social learning of their children. However, the current evidence could not delineat exact pathway. More cross-cultural study should be conducted in this issue.

5.3 Insomnia and psychiatric disorders

Insomnia has been considered to be highly correlated with psychiatric disorders in general population.[172-174] In consistent with previous findings, both phase 1 and 2 studies found that insomnia was associated with psychiatric disorders in the overall participants, especially for middle-age adults.

Only lifetime psychiatric diagnosis had significant heritability in our study. No genetic correlations were found between insomnia disorder and psychiatric disorders when performing bivariate genetic analysis by SOLAR. The negative findings in bivariate genetic analysis for insomnia and psychiatric disorders might be due to limited power when using dichotomized approaches to classify subjects as only few cases were found to have psychiatric disorders in adolescent and father groups. Hence, we performed further genetic analysis for insomnia, depression, anxiety and other

psychological distress by analyzing these symptoms measures as continuous variables.

The phenotypic correlations were quite high between ISI and HADS anxiety (r=0.40) and between ISI and BDI (r=0.44) respectively in overall sample. Bivariate genetic analysis demonstrated that genetic component played an important role on the correlation of insomnia with depressive (p_G=0.56 vs p_E=0.41) and anxiety (p_G=0.51 vs p_E=0.34) symptoms. Hence, our findings suggested that genetic factors appeared to link between insomnia and mood symptoms. The evidence for shared genetic factors by insomnia, depression and anxiety was supported by findings of recent association study that Serotonin Transporter Length Polymorphism (5-HTTLPR) was significantly correlated with primary insomnia[58] and has been found to be associated with depression,[59] albeit the relationship was challenged by a recent meta-analysis which pooled all publications together.[166] The genetic component underlying the relationship between insomnia, depression and anxiety is far from clear.

Furthermore, probands' insomnia could predict lifetime anxiety disorders for their first degree relatives during initial analysis. The association between insomnia of probands and anxiety disorders of first degree relatives might imply that insomnia and anxiety might share common genetic or environmental component. In other words,

these results suggested that there might be co-aggregation phenomenon[175] between insomnia and anxiety disorders. However, the relationship between proband's insomnia disorder and first degree relative's anxiety disorders could not be maintained after controlling for proband's anxiety disorders and first degree relative's insomnia disorder. In fact, due to the limited cases with anxiety disorders in proband group, we could not find heritability for anxiety disorders (table 16). When using continuous variables as assessed by ISI and HADS-anxiety, there was significant phenotypic correlation (p_P=0.40) between insomnia and anxiety symptoms. The correlation was further found to be partially contributed by shared genetic component (p_G=0.51). Hence, our data suggested that insomnia disorder and anxiety disorders might be genetically co-transmitted. Further studies with larger sample size should be warranted in testing the co-aggregation between insomnia and anxiety (further discussion in chapter seven).

5.4 Impact of insomnia on pain and somatic distress

Insomnia was commonly found in patients with pain symptoms caused by other diseases, such as headache, rheumatoid arthritis, fibromyalgia and cancer.[147] Previous studies also found that insomnia at baseline could also predict pain symptoms in

several diseases, such as depression[176] and burn injury.[177] Our phase 1 study also showed that insomnia at baseline was associated with chronic pain that required medical treatment in general population. On the other hand, pain has also been found to predict the incidence of insomnia.[34, 38] Janson et al found that joint/low back pain at baseline could predict the incidence of insomnia after 10 years in men with an OR of 2.95.[34] More recently, LeBlanc et al found that higher body pain was an important predictor for insomnia incidence.[38] Another interesting study with intensive daily measures in both sleep and pain symptoms found that there were day-to-day association between sleep quality and pain in elderly insomniacs although there was no association between average pain and average sleep across 14 days.[178] These prospective studies suggested that the relationship between insomnia and pain should be bidirectional. Our study found that insomnia was correlated with pain and somatic symptoms in both adolescents and adults. Insomniacs had higher scores in nearly all domains of VAS-pain and SSI pain scores and insomnia severity was significantly correlated with VAS-pain and SSI pain scores.

The mechanism underlying the relationship between insomnia and pain is unclear. As discussed above, a possible explanation for the relationship between insomnia and pain might be the reduced SWS related to insomnia. Experimental studies found that

SWS sleep deprivation could reduce mechanical pain threshold, increased pain sensitivity and inflammatory skin flare response.[147] The relationship between insomnia and chronic pain might be mediated by decreased SWS sleep in these patients. Another explanation for this relationship might be the shared genetic component by insomnia and pain. Bivariate genetic analysis in our study demonstrated that the shared genetic component between ISI and SSI pain were quite high.

One might also concern that the relationship between insomnia and pain might be mediated by their common comorbid conditions, such as depressive and anxiety symptoms. Our results showed that ISI could predict SSI scores even after controlling for age, gender, BDI and HADS anxiety scores ($p<0.001$) in both adolescents and adults. These results indicated that it was rather unlikely that this relationship was mediated by mood and anxiety symptoms.

5.5 Impact of Insomnia on somatic symptoms

In the past, insomnia was considered as a symptom secondary to physical diseases. Increasing evidences suggested that insomnia is more like to be comorbid with or

even as a cause for other medical conditions. The investigation on relationship between insomnia and somatic symptoms other than pain is scarce. Using self-developed questionnaire, Kim et al found that all somatic symptoms measured (n=8) in their study were closely correlated with insomnia in Japanese population.[179] Furthermore, the number of somatic symptoms had a dose-response effect on the prevalence of insomnia. In our baseline study, we found that children's general health condition and chronic medical condition as reported by their parents were strongly correlated with insomnia with ORs of 4.76 and 2.56 respectively.[27] Our phase 1 study also showed that insomnia at baseline could predict several physical diseases in both adolescents and middle-age adults after 5 years of follow-up. In a recent study, Roberts et al found that chronic insomnia could predict overall perceived somatic functioning after 12-month with an OR of 1.63-2.62 for different subtypes of insomnia in adolescents.[180] In this regard, insomnia seems to be a predictor for physical diseases. However, none of the studies mentioned above employed a validated questionnaire to assess the somatic symptoms, which might limit its reliability.

Our current study with validated somatic inventory and clinical interview confirmed that insomniacs had higher scores in various somatic symptoms in both adolescents

and middle-aged adults groups. Another interesting finding in our study was the gender and insomnia interaction on SSI total scores and sub-scale scores in adults groups ($p<0.05$), which demonstrated that gender differences in somatic symptom only occurred in those subjects who had insomnia diagnosis. Previous study found that female adults report more frequent physical symptoms than males in both medical patients and community samples.[181-184] In their reviews, Barsky et al proposed several mechanisms underlying gender differences in somatic symptoms, such as biological differences, symptom appraisal, socialization, social role and comorbid depressive and anxiety disorders.[181] Thus, our results suggested another possible pathway that gender differences in the somatic responses to insomnia might contribute to the gender differences in somatic symptoms.

5.6 Relationship of insomnia with personality

Neuroticism has been considered as a predisposing and perpetuating factor in the pathogenesis of insomnia.[185] The role of personality on insomnia is complex and might be bidirectional.[62] Some specific personality features might be due to the consequences of the daytime function impairments in insomnia and in this case the relationship between insomnia and personality is rather a state than trait association.[62]

Our studies found that insomniacs had higher scores in neuroticism in both adolescents and adults. Neuroticism was also correlated with the subjective severity of insomnia as measured by ISI and PSQI in adolescents and adults with insomnia. In other words, neuroticism also influences on how the insomniacs estimate their distress caused by insomnia. As the severity of insomnia symptoms has been suggested as a risk factor for the persistence of insomnia,[35] our data supported the hypothesis that neuroticism plays a role on the genesis and perpetuation of insomnia.[62]

Our genetic analysis further revealed that the phenotypic correlations between ISI and neuroticism and between PSQI and neuroticism were mainly accounted by genetic component (p_G=0.53 and 0.81 respectively) rather than environmental factors (p_E=0.41 and 0.27 respectively). As discussed above, short (s-) allele of 5-HTTLPR has been found to be associated with primary insomnia in German population.[58] However, previous genetic studies on the association between neuroticism and serotonin transporter length polymorphism yielded inconsistent results. Two studies did not find significant association between serotonin transporter length polymorphism and neuroticism[186, 187] while a recent study employed more accurate method in determining Single Nucleotide Polymorphisms (SNPs) in serotonin transporter length found association for SLC6A4 (also named 5-HTT) rs6354 and

rs2020936 (positioned in a different linkage disequilibrium [LD] block about 15.5 kb from 5-HTTLPR) with neuroticism, and these two SNPs was also found to be correlated with depression and anxiety.[188] The s-allele has been found to reduce transcriptional efficiency and hence decrease the expression and function of SLC6A4.[189] Furthermore, the processing style for negative information and emotion has also been found to be modulated by 5-HTTLPR length polymorphisms.[190] Nonetheless, the relatively weak association of 5-HTTLRP with insomnia and neuroticism might not totally account for the whole picture of the robust genetic component shared by insomnia and neuroticism as found in our study. Further studies are needed to investigate other potential mechanism underlying the role of neuroticism on the development of insomnia in terms of environmental, genetic and biological levels.

Although insomniacs had lower extraversion scores in both adolescent and adult groups and had lower agreeableness and conscientiousness scores in adults group, our results only found mild phenotypic correlations between insomnia severity (as measured by ISI and PSQI) and extraversion. Further genetic analysis demonstrated that the relationship between insomnia severity and extraversion was mainly due to environmental factors rather than genetic component. Our results suggested that the

relationship between insomnia and other personality traits, such as extraversion and agreeableness, might be state-dependent rather than trait-dependent.

5.7 Relationship of insomnia with quality of life (QoL)

Quality of life (QoL) has been receiving more and more attention in psychiatric research. QoL was defined as "An individual's perception of their position in life, in the context of the culture and values in which they live and in relation to their goals, expectations, standards, and concerns" (WHOQoL study group).[191] Insomnia was found to be correlated with significant impairments in pervasive domains of QoL and the successful management of insomnia would lead to QoL improvement.[1] Although QoL is considered as a culture-dependent concept, there was a dearth of studies on the impact of insomnia on QoL in Chinese population. Insomnia has been found to have negative impact on QoL in community adults,[192] patients with schizophrenia[174] and cancer[193] in Chinese. However, none of the previous studies using diagnostic criteria to define insomnia cases and none of these studies employed validated questionnaire to assess the severity of insomnia and explore the correlation between insomnia severity and QoL. By using diagnostic criteria in determining insomniac cases, our studies replicated the findings that insomniacs had functioning impairments in several

domains of QoL as measured by SF-36 in both adolescents and adults. Previous study suggested that mood problems or other comorbidities might be important mediators in the relationship between insomnia and poor QoL.[1] Multivariate analysis in this study showed that the impact of severity of insomnia (as measured by ISI) on QoL persisted even after adjusting for depressive and anxiety symptoms, which indicated that depression and anxiety could not account for the association of insomnia and poor QoL. The mechanism underlying the relationship between insomnia and poor QoL is complex. Poor QoL might be a cause or effects of insomnia. In our current study, we could not disentangle the cause-effect relationship between insomnia and poor QoL. Bivariate genetic analysis in our study suggested that the majority of correlated phenotypic correlation between insomnia severity and several domains of QoL (mainly emotional and social functioning) could be explained by genetic component rather than environmental factors.

To our knowledge, this is the first study to investigate the familial aggregation and intergenerational transmission of poor QoL by a family-based study approach. Univariate genetic analysis found that several domains of QoL as measured by SF-36 could be partially accounted by genetic factors, such as energy/fatigue, social functioning, general health and total scores of SF-36. The heritability of QoL might

be mediated by other diseases or traits running in family which also exerted impact on QoL, such as insomnia as found in this study. On the other hand, parents with poor QoL also influence the offspring on behavioral and life-style levels which might contribute poor QoL of their offsprings.

5.8 Strengths and limitations of phase two study

Several strengths are notable in this study. To our knowledge, this is the first study investigating the familial aggregation and heritability of insomnia by a case-control design with detailed clinical interview for both probands and their first-degree relatives. The physicians who interviewed the relatives were blind to the diagnosis of probands. The sample in this study is quite large with satisfactory response rate (82.8%). The detailed assessments in DSM-IV axis I psychiatric disorders and other psychological/functional aspects allowed us to analyze the relationship between insomnia and these problems. Finally, the family-based approach with recruitment of father-mother-proband trios provided us a good dataset to run genetic analysis for phenotypes and their correlates

However, several limitations should be noted in this study. First, despite large sample

in this study, relative low prevalence of DSM-IV axis I psychiatric disorders in this community-based adolescent group limited statistical power to analyze the comorbidity between insomnia and psychiatric disorders. We could only analyze the phenotypic correlations and genetic analysis for insomnia, depressive and anxiety symptoms as measured by self-reported questionnaires. Finally, although our study found that there were common genetic component shared by insomnia and other psychological and functional impairment, the cross-sectional design in phase 2 could not delineate the cause-effect relationship between these traits.

5.9 Conclusions of phase 2 study

Insomnia is a highly heritable disorder with robust familial aggregation. We found significant gene-environment interaction on the pathogenesis of insomnia. Insomnia is highly correlated with depressive and anxiety symptoms, chronic pain, other psychosomatic symptoms and neuroticism personality. The relationship of insomnia with these measures might be partially explained by genetic component. Insomniacs had pervasive impairment in health related QoL and the severity of insomnia was

correlated with decreased QoL.

CHAPTER SIX

SEARCHING FOR BIOLOGICAL MARKERS OF INSOMNIA

6.1 Differences in 3-day subjective and objective sleep parameters in subjects with and without insomnia

Our study found that several issues should be noted in documenting sleep quality and quantity by actigraphy and sleep diary. First, there were good agreements in the estimation of sleep duration but poor agreements in the estimation of sleep quality between actigraphy and sleep diary. Second, the differences in subjective and objective sleep parameters had some age-specific effects. For subjective sleep assessment, only prolonged sleep onset latency was found in insomanics in adolescent group but all parameters indicated that insomniacs had poor sleep quality in adult group. On the other hand, for the actigraphic measures, insomniacs in adult group had slightly longer WASO but similar TIB, AST, SOL and sleep efficiency with non-insomniacs. Insomniacs even had longer TIB and AST than those without insomnia in adolescent group. The utilization of actigraphy in assessments of sleep disorders has been incleasingly popular due to its ambulatory and cost-effective nature. However, the validation of actigraphy in the assessment of insomnia has inconsistent results. In

a recent AASM (2007) report, the recruitment of actigraphy in the assessment of insomnia was graded as "option", which implied either inconclusive or conflicting evidence or conflicting expert opinion based on previous studies.[194] Since the release of report, several studies had been published regarding to the validation of actigraphy in assessment of sleep quality in insomniacs or normal sleeper, and most of these studies reported poor validity of actigraphy in assessing sleep quality. [195-197] Only one study found that actigraphy had good properties in differentiating insomniacs from non-insomniacs in adults.[198, 199] Other 4 studies found that actigraphy had poor agreement with videosomnography or subjective assessment of sleep in both insomnia and community sample.[195-197] Overall speaking, previous studies on validation of actigraphy in assessing subjective sleep quality showed that actigraphy have poor validity (concordance) with subjective measure in sleep quality as found in our study. [198-201]However, actigraphy had been showed to have better agreements with PSG when compared with subjective sleep measures (sleep diary or sleep questionnaire) in both clinical and normal sample. In this regard, sleep parameters as measured by actigraphy seemed to be more accurate in assessing objective sleep quality while sleep parameters as measured by sleep questionnaire seemed to be assessing how satifatory does the subject feel about his/her sleep.

Someren found that the reliability of actigraphy in estimating sleep increased upon increasing the number of recording nights in patients with insomnia (n=10) and dementia (n=12). Hence, he suggested that at least 7 days were required to obtained an acceptable reliability.[202] However, this study only focused on the reliability of the measures rather than validity. Recently, Buysse et al reported that, although no differences in average actigraphic parameters were found between chronic insomniacs (n=61) and normal sleepers (n=31), the night-to-night variability (as measured by standard deviation) did differ in WASO (p=0.0001) and sleep efficiency (p=0.003) with statistical significance between these 2 groups.[196] By using this similar approach, we could only found differences in the standard deviation of WASO between insomniacs and non-insomniacs in adult group. Hence, the validation of of actigraphy in insomnia study needs further investigation.

6.2 Searching for biological markers of insomnia: past and present

For decades, people have been searching for the physiological indicators of poor sleep. The classic study in the field of sleep research conducted by Monroe found that poor sleepers had increased rectal temperature, heart rate, basal skin resistance and phasic vasoconstrictions at 30 minutes prior to and during sleep.[203] This study sparkled a

series of studies on physiological differences between poor sleepers and good sleepers in the following decade, which established insomnia or poor sleep as a valid disorder with physiological changes. By more sophisticated methods in measuring physiological indicators and more precise definition in insomnia, increasing evidence suggested that insomnia is a disorder with physiological hyperactivity during both awakening and sleep. Multiple measures have been applied to measure the physiological activities between insomniacs and non-insomniacs, including heart rate variability, stress hormone level, body temperature, evoked and spectral EEG measures and multiple sleep latency test (MSLT), neuro-imaging, neuroimmunology. However, not a single measure has been consistently found to differ between good and poor sleeper across different studies (see two reviews by Bonnet and Arand[14] and Riemann et al[13] respectively). As pointed out by Bonnet and Arand,[14] most of the previous studies were suffering from limited sample sizes, especially for those studies with statistically negative findings.

The searching for the biological or physiological markers for insomnia is still in its early phase. A biological marker with good reliability and validity for a disorder should have several characteristics, such as measurable, stable, specific and sensitive. Reliability refers to how often we would get the same results if data are collected

repeatedly while validity refers to how well the biological markers will predict the diseases. In this regard, none of the above mentioned measures can serve as good biological marker for insomnia with adequate reliability and validity. Two major concerns are worth noted in the validity of this association of insomnia and biological markers. Insomnia is a waxed and waning disorder with high day-to-day variability in its major phenotypes, such as low sleep efficiency and prolonged sleep onset latency. Whether the association of insomnia with these biological markers is due to the sleep loss (state-dependent) or due to the underlying pathophysiology of insomnia (trait-dependent) is unclear. The phenotypes of insomnia might have poor reliability when being measured once. Some of the previous studies employed one to two nights PSG to explore the association of sleep quality with these biological markers might just find the real-time association (state-dependent) between sleep and biological markers. Second, although insomnia has been considered as a disorder of sleep and wakefulness,[204] the timing of the measure of these biological makers might also influence the results. For example, the differences in HRV between insomniacs and non-insomniacs could only be found in those studies collecting heart rate sample during nighttime but not during daytime (as also seen in our study). Furthermore, many biological makers potentially related to insomnia, such as cortisol and hypocretin, have significant circadian rhythm. The fixed and multiple sampling

methods might be needed to resolve this problem. For example, serial salivary cortisol across several days was considered to be more reliable in measuring the HPA axis activity than single sample.[81]

In addition to the methodological concerns, some factors might attenuate the sensitivity and specificity in the association of insomnia and these biological markers. For example, the comorbidity of psychiatric disorders and medical conditions, which could also change these biological markers, might lead to false positive or negative results. Depression by itself is a disease with hyperactivity in several physiological index as similar to those of insomnia, is highly comorbid with insomnia. Hence, the careful selection of the patients with well documented comorbidities is pivotal. In phase 3 study, we sought to explore the biological makers for insomnia in terms of heart rate variability, 24- hour urinary corisol and serial salivary cortisol.

6.2.1 Insomnia and heart rate variability (HRV)

The heart rate (HR) fluctuates and oscillates around a temporary mean value. HR is controlled by sympathetic and parasympathetic systems. The cyclic and non-cyclic changes in sinus rate over time are defined as heart rate variability (HRV). [205] HRV

has been considered as a physical indicator for the autonomic nervous system (ANS) activity.[205] However, the relationship between insomnia and HRV is inconsistent. Earlier studies found that insomniacs had higher average HR frequency during both sleep and pre-sleep.[15, 206, 207] However, the differences in HR between insomniacs and non-insomniacs could not be replicated in other studies.[208, 209] By employing HRV as an indicator of physical arousal, Bonnet and Arand found that insomniacs had significantly increased low frequency (LF) power and decreased high frequency (HF) power across all stages of sleep as measured by PSG.[15, 210] A recent study further found that LF/HF ratio was significantly correlated with nighttime sleep fragmentation.[211] What should be noted is that the previous studies are limited by small sample size. Our study with large sample size recruited from general population could not find any differences in HRV and HR during awakening between insomniacs and non-insomniacs in both adolescent and adult groups. The relationship between HRV and sleep seems to be limited to nighttime as the changes of HRV could not similarly be found in insomniacs during daytime in other studies.[209, 210] Our results, together with previous studies,[209, 210] suggested that HRV during daytime is probably not a good physiological indicator of insomnia.

6.2.2 Low correlation between salivary cortisol and 24-hour urinary cortisol

Salivary cortisol and 24-hour urinary cortisol concentration were two major indicators for assessment of HPA activity in clinical studies. However, previous studies with small sample sizes found mild to no correlation in cortisol level as measured by these two sampling methods. An early study recruited 21 normal adults volunteers found that there was no correlation between time-integrated salivary cortisol and 24-hour urinary cortisol levels.[212] By employing larger sample size (n=44), Yehuda et al found that there was mild correlation between 24-hour urinary cortisol and waking salivary cortisol ($r=0.32$, $p<0.05$) but not other 5 time points of serial salivary cortisol ($p>0.05$).[213] The correlation between salivary cortisol and 24-hour urinary cortisol was not clear in adolescents. Our study with larger sample size in both middle-age adults and adolescents extended previous knowledge in several aspects. First, consistent with previous studies, we found low to moderate correlation between salivary cortisol and 24-hour urinary cortisol levels in both adolescents and adults. Second, we found that adolescents had stronger correlation between 24-hour urinary cortisol and salivary cortisol than adults, which suggested that this correlation is age-specific. Third, 24-hour urinary cortisol level was correlated with AUCg rather than ACUi of CAR, which indicated that dynamic changes of CAR did not affect 24-hour urinary output of the cortisol.

6.2.3 Relationship of sleep quality and quantity with 24-hour urinary cortisol and serial salivary cortisol

The association of sleep quality and quantity with objective and subjective sleep parameters in both adolescents and adults were summarized in table 61. The association of sleep parameters/insomnia and cortisl assessments found in this study were as follows: 1) Subjective sleep quantity and quality was consistently and negatively correlated with 24-hour urinary cortisol and salivary cortisol levels in adolescents. However, there was no such association in adults. 2) Adolescents with insomnia diagnosis had lower salivary cortisol at 0 minute after waking up (T1) but less decrease in AUCi3 than non-insomniac adolescents. Althougth there was no difference in serial salivary cortisol between insomniacs and non-insomniacs in adult, insomnia diagnosis interacted with gender on the effects of ACUi and salivary cortisol level at 10:00 pm. 3) There was no difference in 24-hour urinary cortisol between insomniacs and non-insomniacs. 4) There were some inconsistent associations of salivary cortisol with objective and subjective sleep parameters between continuous and dichotomized approaches. Fox example, there was no correlation between salivary cortisol and objective sleep measures in adults when using continuous

variables, but, short sleepers as defined by objective TIB≤400 minutes had higher cortisol levels at T1 (13.5±7.9 nmol/L vs 11.2±5.0 nmol/L) and T2 (14.0±6.0 vs 11.5±6.2 nmol/L) than their counterparts (TIB>400 minutes). In brief, cortisol (both salivary and urinary samples) level was more likely to be correlated with subjective measures of sleep than objective measures or insomnia diagnosis. In particular, the association predomninantly occurred in adolescent group.

These results indicated that the relationship amongt insomnia, sleep quantity and quality and cortisol was complex. As discussed in introduction, the relationship between insomnia and HPA axis is equivocal. Several strengths in this study were worth noting. First, the diagnosis of insomnia and psychiatric disorders were confirmed by physician administrated clinical interview. Second, our results were drawn from a relatively large sample size. Thus, we are confident with the ascertainment of diagnosis and statistical power of this study. Third, the serial sampling method across different time points during daytime allowed us to portray the profile of salivary cortisol for individual subjects.

With regards to 24-hour urinary cortisol, there was a dearth in the studies on its relationship with insomnia. To the best of our knowledge, there were only one study

which compared 24-hour urinary cortisol level between insomniacs and non-insomniacs. In consistent with our results, this study also found that there was no difference in 24-hour urinary cortisol between insomniacs and non-insomniacs among patients with chronic fatigue syndrome.[214] Another study with modified sampling method of urine found that there were also no differences in AM (first-voided urine specimen) and PM (3-hour time span before usual bedtime) between insomniac women and normal sleepers, but this study found that AM-PM differences in urinary cortisol level were higher in subjects with insomnia.[215] On the other hand, the current study extended previous knowledge in several aspects in the relationship between 24-hour urinary cortisol and sleep. First, the association of sleep quality with 24-hour urinary cortisol was age-dependent. 24-hour urinary cortisol level was negatively correlated with both subjective and objective sleep duration and was correlated with subjective sleep quality (sleep efficiency and WASO) but not objective sleep quality in adolescents. No association was found between 24-hour urinary cortisol and various objective and subjective sleep parameters in adults. Second, as mentioned above, 24-hour urinary cortisol level did not differ between insomniacs and non-insomniacs and was not correlated with ISI and PSQI.

In summary, there was an emerging pattern of relationship between poor sleep and

elevated cortisol, especially in subjective measures of sleep in adolescent group. The higher the cortisol levels in 24-hour urinary cortisol,waking cortisol and evening cortisol, the shorter sleep duration and poor sleep quality was. However, one should be cautioned when interpreting our results in view of following aspects: 1) These associations were not consistent between adults and adolescents. 2) The relationship of sleep with cortisol was totally different between subjective and objective measures except for the relationship between sleep quanity and 24-hour urinary cortisol level in adolescent group. 3) There was stronger correlation with continuous variables rather thatn dichotomized variables as based on clinical diagnosis. 4) Mutiple statistical comparisons were employed in the sleep and cortisol assessments. 5) Although significant familiality and heritability were found in insomnia disorder, 3-day sleep measures and serial salivary cortisol, there was not consistent association of cortisol levels with sleep measures in the current study. Hence, we did not analyze the bivariate genetic analysis between cortisol and sleep in view of the absence of association.

As mentioned above, a biological marker or endophenotype should have good reliability and validity. In this regard, a single day record of serial salivary cortisol profile or 24-hour urinary cortisol might not be reliable biological markers of

insomnia. The variability of serial salivary cortisol as measured by repeated sampling across several consecutive days might be more precise in analyzing the cortisol profiles.[216] Previous studies found that there were weekday-weekend differences in CAR,[217, 218] and hence, the day of cortisol assessment was suggested to be crucial in stress studies.[218] Due to the sampling requirements for both salivary and urinary sample, all the samples were collected during weekends or holidays in this study. Nonetheless, in assurance of accuracy of the timing of sample collection, we asked the participants to chart down the exact time for each sample. We excluded those samples for estimation of CAR (T1-T4) collected outside a margin of 5 minutes before or after the time protocol. The inconsistent results seem not to be related to the potential sampling error. One might also concern that other comorbidity in this population might attenuate the effects of insomnia or poor sleep on HPA axis activity. In view of the potential confounding effects of psychiatric disorders in the relationship between insomnia/sleep and cortisol level, we excluded those subjects with current psychiatric disorders. Similar results were found in this dataset.

Table 61 Summary of the relationship between sleep measures and cortisol assessments

	Adolescents		Adults	
	Serial salivary cortisol	**24-hour urinary cortisol**	**Serial salivary cortisol**	**24-hour urinary cortisol**
Insomnia diagnosis	↓	--	--	--
Objective measures				
Sleep quantity	--	↓ ↓	±	--
Sleep quality	--	--	--	--
Subjective measures				
Sleep quantity	↓ ↓	↓ ↓	↓	--
Sleep quality	↓ ↓	↓ ↓	±	--

Sleep quantity includes time in bed and actual sleep time

Sleep quality includes sleep efficiency, sleep onset latency and WASO

-- No statistically significant association was found

↓ Negative association (Weak); ↓↓Negative association (strong and/or persistent)

± No consistent relationship between continuous and dichotomized approaches

CHAPTER SEVEN

GENERAL DISCUSSION

7.1 Overall characteristics of this study

This study was a large-scale 3-phase design longitudinal study with fair response rate in phase 1 study (47.2%) but good response rate in phase 2 study (82.8%). To reduce the drop-out rate, repeated mailings and callings were employed in phase 1 study. Only slight differences in socio-demographics were found between those subjects recruited into follow-up study and those who dropped out. The equivalent rate of baseline insomnia between recruited subjects and the drop-outs (adolescents, fathers and mothers) suggested that sample attrition in this cohort was probably limited, albeit the response rate was less than "satisfactory" in phase 1.

In phase 2, a relatively large sample size (236 probands and 447 first-degree relatives) was recruited into comprehensive clinical and psychometric assessments. No differences in socio-demographics were found between insomniac probands and non-insomniac probands except for slightly older age. In this phase, multiple measures were employed to assess the severity of insomnia, psychiatric comorbidity,

psychosomatic distress, quality of life and physical aspects of the subjects. These measures allowed us to analyze different aspects of insomnia and its influences on health.

In phase 3, we tested the relationship of insomnia and sleep parameters with three potential biological markers, HRV, 24-hour urinary cortisol and salivary cortisol, with relatively large sample size in both adolescents and middle-aged adults.

7.2 Genetic analysis for insomnia by father-mother-offspring trios approach

Genetic analysis of complex traits, such as psychiatric and sleep disorders, is relatively difficult as the traits are determined by many genetic and environmental factors. The factors might exert their effects on each individual or even interact with each other. These individual genetic and environmental factors might each contribute a small proportion of effect on the causation of the trait. Familial aggregation as found in previous and current studies suggested that insomnia is potentially a genetically transmitted disorder. However, familial aggregation might also be due to shared environmental factors. Hence, we need to differentiate genetic component from environmental factors. The separation of genetic and environmental effects can be

achieved by twin study, fixed clusters that include a variety of degrees of relatedness (particularly the family set-design), and comparisons of separately reared relatives.[219] In this case, we used a father-mother-offspring approach to calculate the heritability of insomnia and preliminarily analyze genetic and environmental variances. We found that there were significant associations between father-offspring and between mother-offspring pairs but not father-mother pairs in insomnia diagnosis. These results indicated that genetic component plays a critical role in the pathogenesis of insomnia. The employment of father-mother offspring trios approach in this study moves a step forward to analyze heritability beyond familial aggregation. The logic in the genetic analysis by POLAR is as follow: it was assumed that father, mother and offspring shared the same environmental factors liability to insomnia and the weight of the association in genetic factors liability to insomnia was 0.5 for father-offspring and mother-offspring pairs but was 0 for father-mother pair. Estimation of heritability could help us to explore what proportion of the trait variance could be accounted by genetic contribution. The higher the heritability, the higher probability of genetic transmission the trait is. By this conceptual framework,[118] the heritabilities for current and lifetime insomnia as estimated by POLAR analysis were quite high. However, there were two important issues we should be aware before interpreting the heritability estimation by this approach. First, the heritability was narrow sense

heritability such that the phenotypes determined by recessive genes could not be calculated. Second, the heritability might be overestimated if the correlations of parent-offspring pairs were larger than those of father-mother pairs in environmental factors. On the other hand, the heritability might be underestimated in the reverse situation.

7.3 Multifactorial threshold models for familial aggregation of insomnia

In introduction section, we proposed three pathways underlying familial aggregation of insomnia including genetic factors, environmental factors and other comorbid conditions running in family. The genetic analysis in our studies suggested that both genetic and environmental factors accounted for insomnia diagnosis and insomnia symptoms severity. Furthermore, the phenotypic correlation between insomnia, anxiety and depression symptoms also suggested that comorbid conditions were likely involved in the familial aggregation of insomnia. The interaction between parental history of insomnia and life stress on insomnia further demonstrated that environmental stressors have more deleterious effects on those individuals with a genetic predisposition.

Neale and Kendler proposed several threshold models for comorbidity between disorders which are likely contributed by multiple factors.[220] Here, we adapt these conceptual models for insomnia. A threshold model is based on the assumption that the disease's liability is due to the combined effects of independent action of a large number of risk factors, each of which exerts very small effect, and gives rise to a normal distribution of liability. In these models, liability is a latent variable which can not be measured directly. The threshold model assumes that individuals with a combined liability over the threshold are prone to suffer the disease. As mentioned above, the liability of insomnia includes gene, environment, gene-environment interation and effects of other comorbidity. When there is no significant familial clustering of insomnia, there is no correlation in the liability to parental insomnia and offspring's insomnia (figure 14 (a)). Four models (figure 14 (b-e)) might account for significant familial clustering of the diseases.

In the correlated model (figure 14 (b)), the familial clustering of insomnia was due to the correlation of their liability. The strength of the correlation in the liability to insomnia determines the strength of correlation in insomnia between parent and offspring. When there is no correlation (r=0) in liability, familial clustering of insomnia arises by chance. As we found in baseline study and phase 1 follow-up study

that low education is a risk factor for the prevalent and persistence of insomnia in adults and their children. We have also known that the education levels had correlation between spouse pair. Hence, co-occurrence of insomnia between parents and children could be due to the correlation between their education levels.

In the interactive model (figure 14 (c)), the familial clustering of insomnia was due to the effects of liability to insomnia on the other one's insomnia. For example, we found that children's medical and behavioral problems was correlated with not only their insomnia but also their parents' insomnia.[27] In this situation, if the effects of children's medical and behavioral problems on parental insomnia were not mediated by the liability to parental insomnia, then interactive model is appropriate for explanation of familial aggregation of insomnia. Co-aggregation of insomnia and other disease can also be considered as a special case in the interactive model. As we know that one of the common comorbidities of insomnia is anxiety disorders and both insomnia and anxiety have significant familial aggregation phenomenon. In statistical model, if parental anxiety disorders could predict offspring's insomnia independent of parental insomnia and offspring's anxiety disorders, we would draw the conclusion that co-aggregation exists in these two disorders.[175] In our study, we found that probands' insomnia could predict anxiety disorders in first degree relatives with an OR (95%CI)

of 1.93 (1.04-3.58) in initiatial analysis. However, this association could not be maintained after adjusting for proband's anxiety disorders and first degree relatives' insomnia ($p>0.05$). Larger sample size in future study might be appropriate.

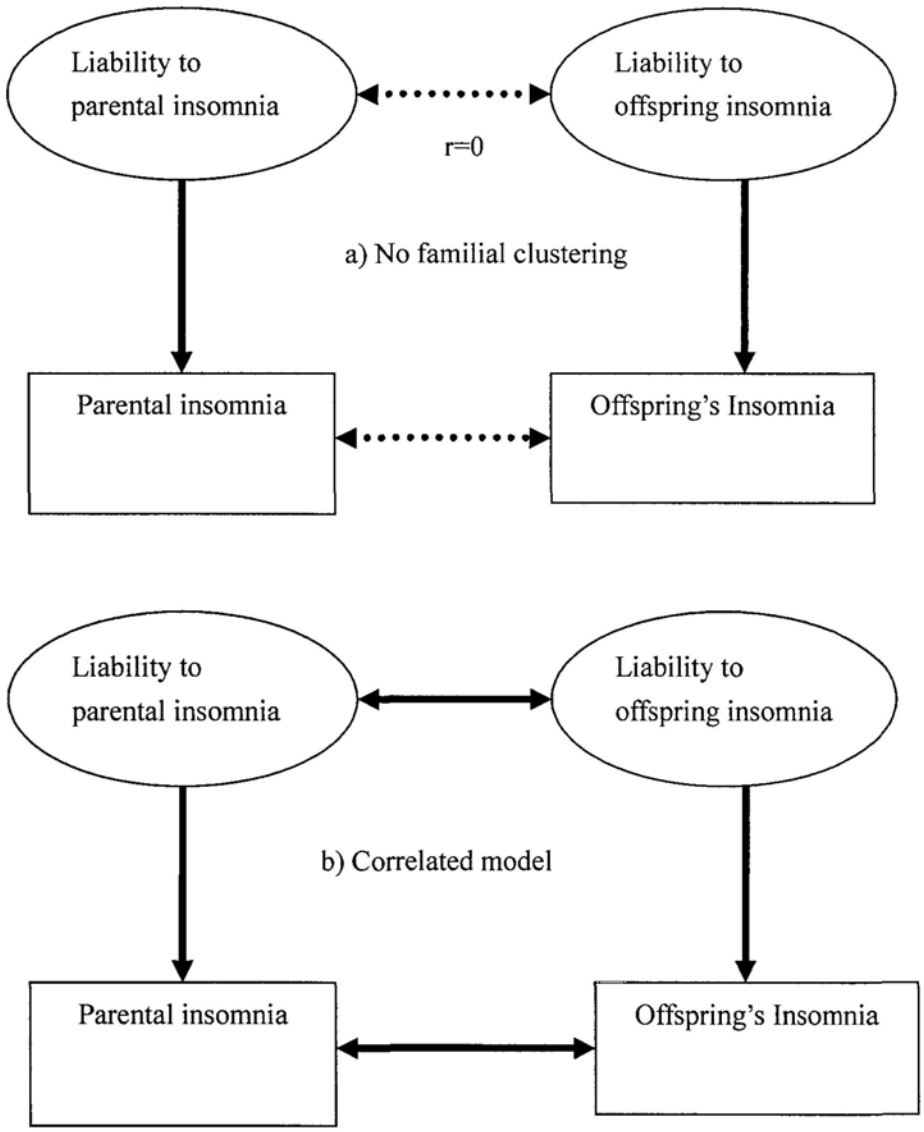
In the overlapped model (figure 14 (d)), the familial aggregation of insomnia is due to the overlapping of the liability to insomnia among family members. Indeed, several factors are definitely overlapped between parents and offspring, such as socio-economic status (especially for early life of offspring) and genes.

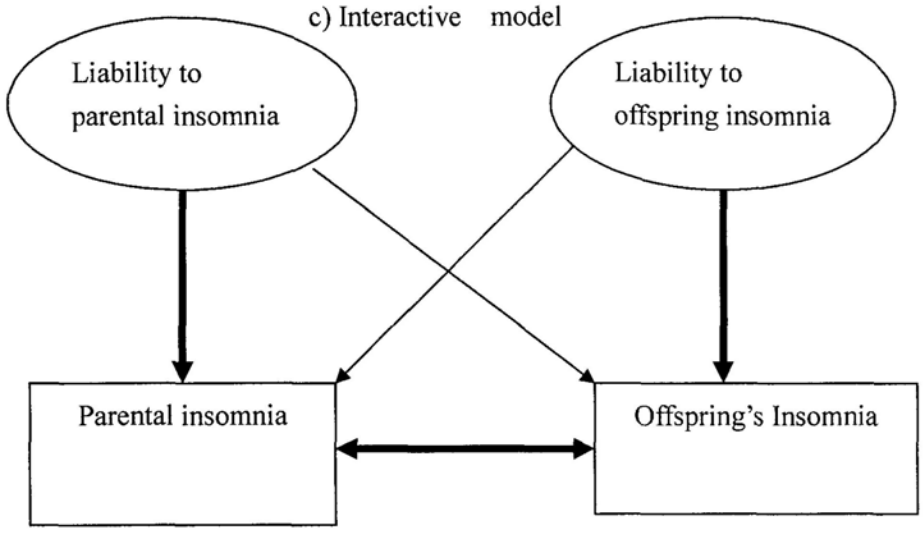
In the independent model (figure 14 (e)), insomnia is divided into sporadic insomnia and familial insomnia. The typical one is the fatal familial insomnia (FFI), which is a prion disease related to mutation at codon 178 (D178N) of prion protein (PrP) gene (*PRNP*).[57] However, there were only very few cases found in worldwide, which indicated that this disease (FFI) could not explain most of the insomnia cases. To explore independent model for general cases of insomnia, the first step is to compare the differences between sporadic cases and familial cases in terms of socio-economic status, symptom severity, comorbidity, psychometric measures (such as personality) and also biological markers (although negative results found in preliminary analysis). Our future analysis should further delineate the differences of above mentioned socio-

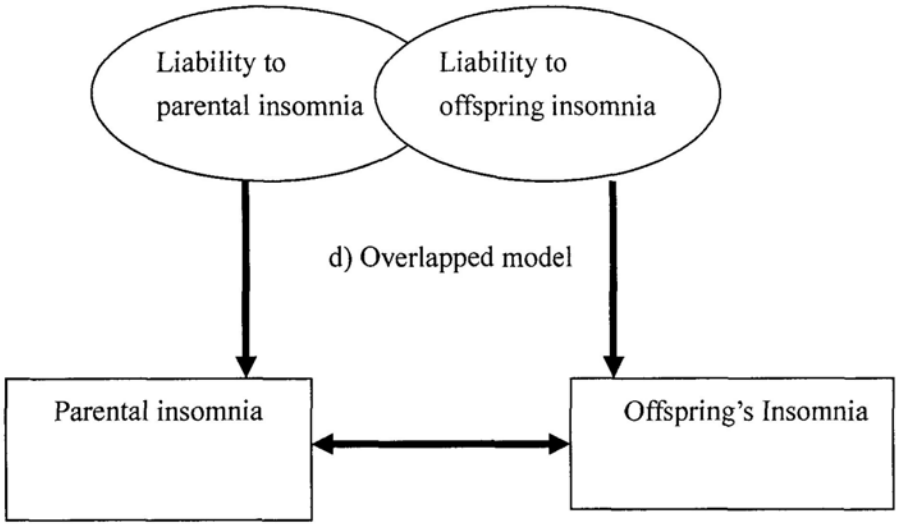
economic status, psychometric measures and also biological markers between these two subtypes of insomnia, sporadic and familial insomnia. However, it is difficult for us to differentiate overlapped model from independent model in a cross-sectional study even if we find differences between sporadic insomnia and familial insomnia.

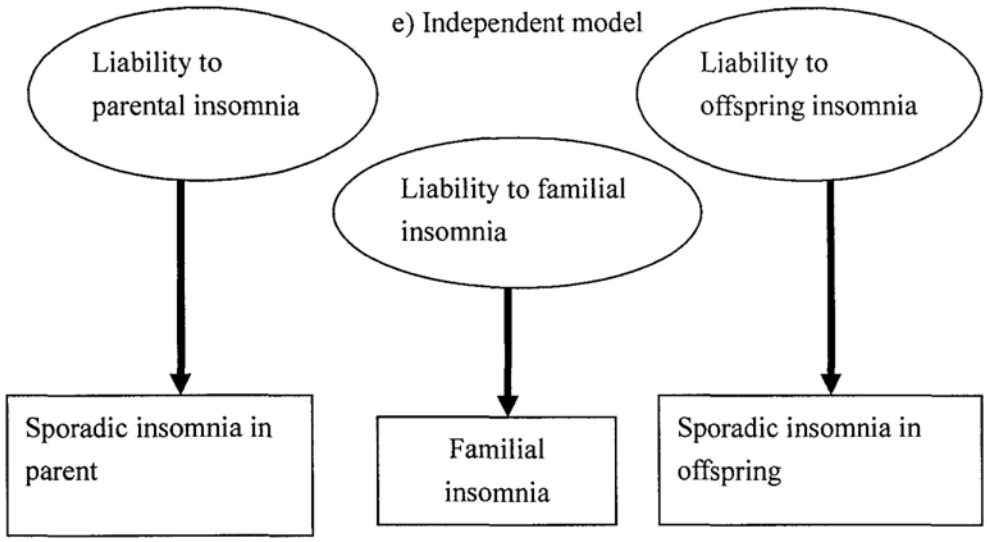
Overall speaking, our results preliminarily suggested that not a single model out of the four above mentioned models could explain all aspects of familial aggregation of insomnia. The confirmation of any model in elucidating familial aggregation of insomnia should be tested in a sufficient sample size study with good quality of design such as longitudinal family or twin study with simultaneous documentation of psychometric, mental and medical comorbidities of insomnia. Further in-depth study with hypothesis driven design might provide more evidences for the determination of general models in delineating the mechanism underlying familial clustering of insomnia.

Figure 14 Conceptual models for familial aggregation of insomnia




c) Interactive model
Liability to parental insomnia
Liability to offspring insomnia
Parental insomnia
Offspring's Insomnia


Liability to parental insomnia
Liability to offspring insomnia
d) Overlapped model
Parental insomnia
Offspring's Insomnia


e) Independent model
Liability to parental insomnia
Liability to familial insomnia
Liability to offspring insomnia
Sporadic insomnia in parent
Familial insomnia
Sporadic insomnia in offspring

7.4 Implications of this study

7.4.1 Insomnia is a persistent disorder, but how persistent is it in Chinese population?

This is the first study investigating the long-term course of insomnia in Chinese general population. Several findings are worth noting in our study. First, insomnia tends to persist in Chinese population with age- and gender-specific relationship. The gender differences in insomnia were due to higher persistence of insomnia rather than higher incidence rate in females. The findings of risk factors accounting for persistence and incidence of insomnia also have important implications for the prevention and treatment of insomnia. Second, in view of high remission rate, insomnia is probably a waxed and waning condition in childhood. Nonetheless, children with insomnia at baseline had higher risk to have insomnia at follow-up than those children without insomnia at baseline with an OR (95%CI) of 2.64(1.31-5.35). Childhood insomnia also predicted several medical outcomes after 5 years, such as frequent asthma and allergic rhinitis episodes. The long-term consequences of insomnia argue for early and long-term intervention.

7.4.2 Treating insomnia comprehensively for both mental and physical reasons

Our study found that baseline insomnia was associated with several common medical conditions (upper airway imflammatory diseases) in both adolescents and adults. Furthermore, we found that insomnia was associated with several chronic medical conditions (such as chronic pain and hypertension) in adults. In the past, insomnia was considered as a pure mental distress. In recent decade, increasing attention has been paid to the medical consequences of chronic insomnia. Our findings that insomnia is associated with pervasive medical complications indicated that insomnia is an independent sleep disorder with both medical and mental consequences. The treatment of insomnia is far from adequate in Chinese population,[174] which may be due to several factors, such as the unwanted side effects, its waxed and waning course and unawareness of its significance in both physicians and patients. Physicians should be aware of epidemiology, diagnosis and treatment of this common disorder in both community and medical settings.

7.4.3 Familial aggregation of insomnia: implications for prevention and treatment

The successful management of maternal depression has been found to benefit the mental health in the next generation.[221, 222] Hence, the findings of familial aggregation of the diseases has been considered to have value for the prevention of depression.[45] The robust familial aggregation of insomnia found in this study also suggested that the improvement of insomnia symptoms in one might also benefit other family members. High first degree relatives' recurrent rate (41.7% for current insomnia) of insomnia found in our studies also suggested that the family-based therapy might also have good theoretical and practical support in the clinical management of insomnia. In this regard, further studies should investigate the effect of family-based treatment on insomnia. Strong familial aggregation, high heritability and gene-environment interaction of insomnia as found in our study also suggested that multi-dimensional management for insomnia might have more efficacy.

7.5 Searching biological markers for insomnia, what should be learned from this study?

Overall speaking, the findings of negative results in searching biological markers for insomnia were disappointing. We did not find a consistent and reliable biological marker for insomnia in terms of 5-minute daytime HRV, 24-hour urinary cortisol and

serial salivary cortisol across different age groups. Furthermore, we found that the objective measures by actigraphy of sleep were not of good validity in differentiating insomniacs from non-insomniacs. Although there was some associations of 24-hour urinary cortisol and serial salivary cortisol with sleep parameters, majority of these associations were in the subjective sleep parameters in adolescent group. Althogh we found that there were some significant associations between insomnia/sleep and salivary cotisol level, these results were limited by multiple comparisons. Our study found modest association between 24-h urinary cortisol and sleep duration (both subejective and objective) and sleep quality (subjective) but not between 24-h urinary cortisol and insomnia, which suggested that the association between sleep and 24-h urinary cortisol was more likely to be state-dependent rather than trait-dependent. In addition, the strength of association was also different between adolescents and adults. In other words, cortisl is unlikely to be a stable biological marker (endophenotype) of insomnia.

In light of high heritability of insomnia found in our study, we believe that there should be genuine biological mechanism underlying the pathogenesis of insomnia, albeit our odyssey of searching biological markers for insomnia was disappointing. More sensitive and reliable test should be employed to investigate the underlying

biological markers for insomnia, such as challenge test for cortisol response, neuroimaging and 24-hour HRV. On the other hand, it should also be noted that the sleep parameters as measured by 3-day actigraphy and questionnaire might not be reliable enough to document severity of insomnia. Finally, the findings of significant association between sleep quality and biological tests from previous studies (especially for those without control group) might probably indicate the real-time association between sleep parameters and state-dependent biological tests rather than the association between insomnia and trait-dependent biological test.

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