STRESS AND OBSTRUCTIVE AIRWAY DISEASES: ASSOCIATION AND THE MEDIATING ROLE OF NEUROPEPTIDE Y

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DECLARATION

I hereby declare that the thesis is my original work and it has been written by me in its entirety.

I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

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25 January 2014

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LIST OF PUBLICATIONS

1. **Lu YX,** Ho RC, Lim TK, Kuan WS, Goh DY, Mahadevan M, Sim TB, van Bever HP, Larbi A, Ng TP. Neuropeptide Y may mediate psychological stress and enhance Th2 inflammatory response in asthma. J Allergy Clin Immunol. 2015; in press.

2. **Lu YX**, Feng L, Feng L, Nyunt MS, Yap KB, Ng TP. Systemic inflammation, depression and obstructive pulmonary function: a population-based study. Respir Res. 2013;14:53.

3. **Lu YX,** Mak KK, van Bever HP, Ng TP, Mak A, Ho RC. Prevalence of anxiety and depressive symptoms in adolescents with asthma: a meta-analysis and meta-regression. Pediatr Allergy Immunol. 2012;23:707-15.

4. **Lu YX,** Ho RC, Lim TK, Kuan WS, Goh DY, Mahadevan M, Sim TB, Ng TP, van Bever HP. Psychiatric comorbidities in Asian adolescent asthma patients and the contributions of neuroticism and perceived stress. J Adolesc Health. 2014;14:1-9.

5. **Lu YX,** Feng L, Lim L, Ng TP. Asthma, life events and psychiatric disorders: a population-based study. Soc Psychiatry Psychiatr Epidemiol. 2013;48:1273-82.

6. **Lu YX,** Nyunt MS, Gwee X, Feng L, Feng L, Kua EH, Kumar R, Ng TP. Life event stress and chronic obstructive pulmonary disease (COPD): associations with mental well-being and quality of life in a population-based study. BMJ Open. 2012;2.

7. **Lu YX,** Tang C, Liow CS, Ng WN, Ho SH, Ho RC. A regressional analysis of maladaptive rumination, illness perception and negative emotional outcomes in Asian patients suffering from depressive disorder. Asian J Psychiatr. 2014;12:69-76.

8. **Lu YX,** Ho RC, Lim TK, Kuan WS, Goh DY, Mahadevan M, Sim TB, van Bever HP, Larbi A, Ng TP. Obesity, inflammatory cytokines, adiponectin and neuropeptide Y: associations with asthma prevalence and Th2-cytokine (interleukin-4) marker of allergic airway inflammation. To be submitted.

9. **Lu YX,** Ho RC, Lim TK, Kuan WS, Goh DY, Mahadevan M, Sim TB, van Bever HP, Larbi A, Ng TP. Neuropeptide Y polymorphism and adiposity in asthma during a one-year prospective follow-up. To be submitted.

CONTENTS

ABSTRACT

Background: The prevalence of obstructive airway diseases such as asthma and chronic obstructive pulmonary disease (COPD) has increased significantly in recent decades, concurrently with increasing mental health problems worldwide. However, the underlying mechanisms especially the relative contribution of psychological stress to the psychiatric co-morbidities and functioning impairment observed in obstructive airway diseases are not well elucidated.

Objective: This study aims to explore the stress-airway obstruction association in adolescent, adult and elderly individuals, and the underlying role of neuropeptide Y (NPY) through a cross-sectional clinical study, a clinical follow-up study and population-based studies.

Methods: A systematic meta-analysis and meta-regression was performed about the prevalence of anxiety and depressive symptoms in adolescents with asthma in Study I. The stress-asthma link was investigated in a clinical study (Study II) of adolescents with well controlled asthma (n = 137), poorly controlled asthma (n = 61), and healthy neighbourhood controls (n = 171). Questionnaires were administered to explore the symptom profile of specific anxiety and depressive comorbidity (panic attacks, social phobia, generalized anxiety, obsession and compulsion, separation anxiety, depression, total anxiety and total internalizing symptoms (anxiety and depressive)) in adolescents with asthma and the role of perceived stress in explaining the association between asthma and psychiatric comorbidity. In Study III, data in a nationally representative sample of Singaporean adults aged 20-59 (n = 2847) were analysed for asthma, other chronic physical conditions (e.g., coronary heart disease, stroke, lipid abnormalities), and no chronic physical conditions. Participants were assessed for stressful life events, psychiatric disorders, and quality of life. Population-based data were analysed for a sample (n = 497) of older persons aged 65 and above (Singapore Longitudinal Ageing Study (SLAS), Study IV) with airway obstruction (post-bronchodilatation

FEV1/FVC < 0.70, n = 136) or without airway obstruction (n = 277). The main effects of stressful life events and airway obstruction, and their interaction, on measures of pulmonary function, depressive symptoms (Geriatric Depression Scale), cognitive function (Cognitive Failures Questionnaire and Mini-Mental State Examination), and quality of life were investigated. Study V investigated the association of measures of psychological and biological stress with T helper cell type (Th)2 expression of Interleukin-4 (IL-4), a cytokine marker of allergic airway inflammation in asthma and the potential mediating role of NPY in this association among 70 young adult (21-35 years old) acutely exacerbated and non-exacerbated chronic asthma patients, and 69 age- and gender-matched healthy controls. The participants were assessed for the levels of perceived stress (Perceived Stress Scale), hypothalamo-pituitary-adrenal (HPA) hormones, adrenocorticotropic hormone (ACTH), adrenaline (A), noradrenaline (NA), cortisol, NPY and IL-4 (measured at baseline and 12-month follow-up).

Results: Study I showed that the aggregate prevalence of depressive and anxiety symptoms was significantly higher among 3,546 adolescents with asthma than that of 24,884 healthy controls (Depression: 0.27; 95% CI: 0.18.6-0.39 vs. 0.13; 95% CI: 0.09-0.19; Anxiety: 0.33; 95% CI: 0.19-0.52 vs. 0.21; 95% CI: 0.12-0.33). The risk of developing depression and anxiety was significantly higher among adolescents with asthma when compared with the healthy controls (depression: OR = 2.09, p < 0.001; anxiety: OR = 1.83, p < 0.001). Meta-regression revealed that the proportions of Caucasian (p < 0.01) and smokers (p < 0.001) were significant moderators which explained the significant heterogeneity when comparing the risk of developing depressive symptoms among adolescent asthma patients versus the healthy controls while age, gender and severity of asthma were not significant. In Study II, adolescents with poorly controlled asthma, compared with well controlled asthma patients and the healthy controls, had higher scores of depression (p = 0.006), panic attacks (p = 0.002), total anxiety (p = 0.038) and total internalizing symptoms (p = 0.017), as

well as perceived stress (p = 0.022), after adjusting for potential confounders. Perceived stress explained to a great extent the psychiatric comorbidity scores both in the whole sample and in asthma patients. Study III found that the asthma group reported significantly more stressful life events than individuals with other chronic medical conditions (OR = 2.93) and the healthy controls (OR = 4.88). Among individuals with asthma, stressful life events contributed significantly towards increased psychiatric comorbidity and worse SF-12 Mental Component Summary functioning scores. In the elderly (Study IV), stressful life events were found to be associated with more depressive symptoms (Main effects $_{\text{stress}}$: F = 64.500, $p < 0.001$; Main effects $_{\text{airway obstruction}}$: F = 2.353, p = 0.126; Interaction: F = 10.970, p = 0.001) and worse physical (Main effects $_{\text{stress}}$: F = 7.054, p = 0.008; Main effects $_{\text{airway obstruction}}$: F = 0.432, p = 0.512; Interaction: F = 4.055, p = 0.045) and mental functions (Main effects $_{\text{stress}}$: F = 14.710, $p < 0.001$; Main effects $_{\text{airway obstruction}}$: F = 0.659, p = 0.417; Interaction: F = 4.538, p = 0.034) in participants with airway obstruction than in those without airway obstruction after adjusting for potential confounders. In Study V, higher levels of perceived stress, corroborated by elevated levels of ACTH, NA, A, derived HPA stress index, and depressed cortisol were observed in patients with asthma than with healthy controls ($p < 0.05$). NPY levels congruent with chronic stress exposure was lower in asthma patients versus controls (p = 0.01). Among asthma patients, perceived stress and NPY were significantly and positively associated with elevated IL-4 levels at baseline and 1-year follow up. NPY significantly mediated the association of psychological stress with IL-4 (Sobel tests: p = 0.033, baseline IL-4; p = 0.032, IL-4 one year later). The HPA index measure of transient biological stress, independent of NPY, was a significant predictor of IL-4 at baseline but not at one-year follow up.

Conclusions: Results of the present thesis suggest the pivotal role of psychological stress to the association of psychological symptoms and obstructive airway diseases. NPY may be a plausible

neuroendocrine mediator for the persistent effect of perceived stress on heightening Th2 immune and inflammatory responses in asthma, and a candidate for early targeted interventions.

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LIST OF ABBREVIATIONS

A	Adrenaline
AAI	Allergic airway inflammation
ACT	Asthma Control Test
ACTH	Adrenocorticotrophic hormone
ANCOVA	Analysis of covariance
ANOVA	Analyses of Variance
APCs	Antigen-presenting cells
AQLQ	Asthma Quality of Life Questionnaire
ATP	Adenosine Triphosphate
ATS	American Thoracic Society
BMI	Body Mass Index
CBT	Cognitive behavioural therapy
CFQ	Cognitive Failures Questionnaire
COPD	Chronic obstructive pulmonary disease
CRH	Corticotropin-releasing hormone
CSF	Cerebrospinal fluid

df	Degree of freedom
DHEA	Dehydroepiandrosterone
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
DSRB	Domain Specific Review Board
ELISA	Enzyme-linked immunosorbent assay
EMD	Emergency Medicine Department
FeNO	Fractional exhaled nitric oxide
FEV1	Forced expiratory volume in 1 second
FFI	Five-Factor Inventory
FFM	Five-Factor Model
FVC	Forced vital capacity
GAD	Generalized anxiety disorder
GDS-15	15-item Geriatric Depression Scale
GHQ	General Health Questionnaire
GOLD	The Global Initiative for Chronic Obstructive Lung Disease
HADS	Hospital Anxiety and Depression Scale
HPA	Hypothalamo-pituitary- adrenocortical

ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
IL	Interleukin
LTB4	Leukotriene B4
LTEQ	List of Threatening Experiences Questionnaire
MCS	Mental Component Summary
MDD	Major depressive disorder
MMSE	Mini-Mental State Examination
mRNA	Messenger ribonucleic acid
NA	Noradrenaline
NHLBI	National Heart Lung and Blood Institute
NMHS (A)	National Mental Health Survey (Adults)
NPY	Neuropeptide Y
NUH	National University Hospital
OR	Odds ratio
PAQLQ	Paediatric Asthma Quality of Life Questionnaire
PASW	Predictive Analytics Software

PCS	Physical Component Summary
PSS	Perceived Stress Scale
PTSD	Posttraumatic stress disorder
QoL	Quality of life
RCADS	Revised Child Anxiety and Depression Scale
RCCM	Respiratory and Clinical Care Medicine
SAM	Sympathetic-adrenomedullary
SCAN	Schedule for Clinical Assessment in Neuropsychiatry
SE	Standard error
SF-12	Medical Outcomes Study 12-item Short Form
SF-36	Medical Outcomes Study 36-item Short Form
SLAS	Singapore Longitudinal Ageing Study
SNP	Single nucleotide polymorphism
SRH	Self-rated health
SRRS	Social Readjustment Ratings Scale
TGN	Trans-Golgi network
Th	T helper cell type

TNF-α	Tumor necrosis factor alpha

CHAPTER 1 INTRODUCTION

Asthma affects over 300 million people worldwide. Chronic obstructive pulmonary disease (COPD) will rank the fifth in the global burden of disease by 2020. [1] The increased prevalence of obstructive airway diseases is occurring concurrently with increasing mental health problems and impaired quality of life. The connection between stress and airway obstruction has vast aetiological significance, and may represent the next leap in advancing knowledge about airway disease aetiology, prevention and treatment. This thesis focused on aspects of the contribution of psychological stress to the psychiatric comorbidity of airway obstruction in different age groups including adolescent, adult and the elderly individuals, as well as the role of neuropeptide Y (NPY) in mediating the persistent effect of perceived stress on heightening T helper cell type (Th)2 immune and inflammatory responses in asthma. Research into the role of neuropeptides in the inter-individual variability in stress-airway obstruction connection will greatly enhance the understanding of asthma pathophysiology from a psycho-neuro-immunological perspective. Chapter 1 presented the general background and context of the current study. More detailed literature review was covered in Chapter 2.

1.1 Obstructive airway disease and its burden to society

Obstructive airway diseases such as asthma and COPD are a category of respiratory diseases characterized by airway obstruction. It is generally distinguished by inflamed and easily collapsible airways, airflow obstruction, problems with exhaling and frequent physician and Emergency Medicine Department (EMD) visits and hospitalizations. [2] Obstructive airway disease has shown increasing prevalence in various populations. Among the adult population, the reported prevalence of

asthma varies from 1.9% in Greece to 18.4% in Scotland. [3] In the United States, the mortality for COPD doubled since 1970 to 2002 and currently ranks the fourth leading cause of death. [4] In Singapore, the prevalence of asthma is 10.2% in children and 11.9% in adolescents. [5] COPD accounts for 4.7% of all deaths and 1.2% of all hospitalizations, which is much higher than in the United States, Canada and other countries. [6]

Asthma and COPD pose significant psycho-social, economic and health care burdens to the patients, their families and the society. [3, 7] Notably, the concurrent poor mental health of patients with airway obstruction has received growing attention in recent decades, as increasing numbers of studies [8-10] have reported that psychological factors, particularly anxiety and depressive symptoms, are even better predictors of obstructive airway disease-related functional impairment and quality of life (QoL) than lung function. Patients with severe airway obstruction experience various difficulties with emotional functioning, sleep and rest, physical mobility, social interaction and daily activities. Notably, extensive literature describes the extreme stress and anxiety provoking experience of dyspnea in asthma and COPD. [11, 12] During exacerbation episodes, feelings of suffocating, strangling or drowning are associated with heightened emotions, growing panic, extreme fear, muddled thoughts, and decreased physical energy. [11] The increased prevalence and severity of airway obstruction amidst the background of increasing mental health problems call for research into the relationship between stress and obstructive airway diseases.

1.2 Psychiatric comorbidity in obstructive airway disease

Among obstructive airway diseases, asthma has historically long been considered as a typical psychosomatic disorder. [13] Asthma, along with other obstructive airway diseases, is associated with a high comorbidity with psychiatric disorders such as anxiety, depression, panic attacks, and posttraumatic stress disorder (PTSD). [14-16] Vazquez et al. [17] found that patients with near-fatal asthma showed higher psychological morbidity, notably anxiety, even years after the near-fatal asthma episode. Among adults with asthma and COPD, the co-occurrence of an anxiety or depressive disorder is associated with adverse outcomes such as poor symptom control, impaired QoL, and increased health care utilization. [18, 19]

The mechanisms underlying the psychiatric comorbidity in asthma and COPD are not well understood. [20, 21] It remains unclear to what extent individual psychiatric comorbidity results uniquely from specific or common biological responses, or from psychological factors such as environmental stress and poor coping which may reduce psychological dysfunction. In particular, the relative contribution of psychological stress to the aetiology of psychiatric comorbidities in asthma and COPD is not fully investigated.

1.3 Psychological stress and airway obstruction

Patients with asthma and COPD may experience various stressful life events and psychosocial adversities such as withdrawal from family or social life, social isolation, inability to work, unemployment, interpersonal problems, low self-esteem, financial loss, poor mental health, and reduction of social functioning and life satisfaction. With increased understanding of the

neurobiology of stress and the pathophysiology of airway diseases, elucidating the role of psychological stress in the development and control of airway obstruction is now distinctly possible.

There is marked inter-individual variability in responses to psychological stress. Some individuals are more vulnerable, while others are more resilient. This is likely to be determined by the interaction of genetic and environmental factors, and the timing and duration of stress exposure. Among these factors, one potent determinant may be found in neuropeptides released by neurons that act as neuronal signalling molecules and modulate the release of neurotransmitters and hormones. [22] NPY, which is the most abundant neuropeptide in human brain and is involved in multiple physiological activities such as vasoconstriction, control of food intake and bodyweight, regulation of emotional response, is suggested to play an important role in explaining inter-individual variation in resilience to psychological stress. [23]

1.4. Objectives of the current study

Stressful life events, and how it is perceived and appraised, can have extensive impact on individuals. The contribution of psychological stress to the psychiatric comorbidities and functioning impairment observed in individuals with obstructive airway diseases has not been well elucidated. Research on the role of NPY as a resilience factor in the inter-individual variability in responses to stress is just emerging. In an attempt to fill this knowledge gap, the current study aimed to assess the contribution of stressful life events and perceived stress in the psychiatric comorbidity and quality of life of asthma and COPD in adolescent, adult and elderly individuals, as well as the role of NPY in explaining the underlying neuroendocrine mechanism of this relationship. Accordingly, several

related but independent studies were conducted. For ease of reference, the studies were referred as Study I, Study II, Study III, Study IV and Study V respectively throughout the thesis. Study I was based on literature search and meta-analysis of published existing data. During the literature review, we found that eligible studies were mainly conducted in western countries and research in Asian samples was lacking. Study II-Study IV were performed to explore the stress-airway obstruction association and its health impact in different age groups of adolescent, adult and elderly individuals respectively. Finally, Study V was conducted to investigate the role of NPY in mediating stress-airway obstruction link.

We hypothesized that:

1. Obstructive airway diseases would be associated with a high prevalence of anxiety and depressive symptoms and impaired QoL.

2. More stressful exposures in the past and high concurrent levels of perceived stress might be found in individuals with airway obstruction compared to the healthy controls, and among poorly controlled patients or patients with acute exacerbations, compared to those in well-controlled or stable state.

3. Stressful life events and perceived stress would contribute significantly towards increased psychiatric comorbidity and impaired QoL among individuals with airway obstruction, especially in poorly controlled patients and patients with acute exacerbations.

4. Asthma would be associated with higher levels of psychological stress in young adult participants, corroborated by elevated levels of hypothalamo-pituitary-adrenocortical (HPA)-related levels of adrenocorticotrophic hormone (ACTH), noradrenaline (NA), adrenaline (A), and depressed levels of cortisol and NPY, reflecting blunted adaptive responses to chronic stress.

5. Higher levels of psychological stress (perceived stress)/biological stress (HPA index) may be associated with increased levels of interleukin (IL)-4, and this is likely to be mediated by the levels of NPY.

This series of studies investigated the relationship between stress and psychiatric comorbidity in obstructive airway diseases among different age groups of an Asian population. These studies would contribute to the understanding of the role of stress in obstructive airway diseases, and provide the rationale for identifying key vulnerability and resilience factors for psychiatric comorbidity and screening patients for psychological treatment. Hopefully, the findings from this study may facilitate the design of future clinical trials and the establishment of a prevention and intervention framework for psychiatric comorbidity in asthma and COPD. Research into the role of NPY in inter-individual variability in stress-asthma link may enhance the understanding of psychological stress and asthma pathophysiology from a psycho-neuro-immunological perspective.

CHAPTER 2 LITERATURE REVIEW

Obstructive airway diseases such as asthma and COPD are global public health problems which pose huge societal burden. As a prominent risk factor, the role of psychological stress in airway obstruction deserves much attention. The following literature review focuses on existing research on the link between stress and asthma and COPD, as well as the role of NPY as a resilience factor in the inter-individual variability in stress responses that is related to this thesis.

2.1 Psychological stress and airway obstruction

In previous studies, psychological stress is reported to be closely related to both asthma and COPD. However, the underlying mechanism remains unclear.

2.1.1 Psychological stress

Psychological stress can be defined as the non-specific psychophysiological reaction of the body to a variety of emotional and physical stimuli that threaten the body's homeostasis. [24] Excessive psychological stress may lead to depression, psychological burnout, and PTSD in all age groups, as well as cognitive impairment in the elderly. [25] Stress is also a prominent risk factor for the development and adverse outcomes of chronic illnesses such as type 2 diabetes, coronary heart disease, gastroenterological disorders and obstetric outcomes. [26]

Psychological stress is caused by stressors and one common measure of stress is, therefore, stressful life events. Stressful life events contribute to anxiety and depressive symptoms and disorders. [27, 28]

Studies found that 50%-80% of depressed persons experienced at least one major life event during 3-6 months preceding the onset of depression, while the frequency of experience of a major life event evaluated at the same period was only 20%-30% in non-depressed persons. [29] Stressful life events are closely linked to the development of a wide range of physical diseases as well, in particular cardiovascular disorders, infections, autoimmune diseases, cancers, and obstructive airway diseases. [30]

Although stressors are highly associated with mental and physical health problems, there are considerable inter-individual differences in vulnerability and resilience to potential pathogenic effects of stress. Stress is a subjective response composed of cognitive assessment and emotional reactions. According to Lazarus's cognitive-motivational-relational theory, [31] there is a continual interplay between mediators such as subjective appraisals of stressful life events, coping strategies, and responses such as emotional reactions. Different emotions are elicited when situations are evaluated differently. Perceived stress, a widely used measure of the subjectivity of stress, refers to the feelings or thoughts that an individual has about how much stress they are under at a given point in time or over a given time. This measurement has been shown negatively correlated with self-rated health status, and self-esteem of healthy volunteers and hospital inpatients, and positively correlated with health complaints, anxiety and depressive symptoms, susceptibility to common cold in healthy adults, and emotional exhaustion. [32-36]

Studies suggest that individuals with certain personality traits, such as neuroticism, are more vulnerable to both stress and asthma than their counterparts. [37, 38] Neuroticism is a personality trait

manifested as the tendency to experience negative and distressing emotions, and is reportedly linked to psychiatric morbidity including anxiety, depression and stress susceptibility, as well as exacerbation of asthma. [39, 40] Prior research has suggested that neuroticism is negatively correlated with general health, well-being and negative perception of asthma symptoms. [37, 38] Recurrent unpredicted attacks of asthma, especially poorly-controlled asthma, make patients more neurotic and stressful; thus induce anxiety and depressive symptoms in patients with asthma. [40]

2.1.2 Psychological stress and asthma exacerbation

Asthma is an archetypal psychosomatic disease which is closely related to psychosocial or emotional factors, particularly stress. Historically, asthma was referred to as asthma nervosa of presumably psychogenic origin before the understanding of its underlying inflammatory and immunological mechanisms. In the 1930s and 1940s when asthma had been recognized as a chronic inflammatory disease, leading physicians noticed that parental stress and the quality of mother-child interaction could improve or exacerbate symptoms of childhood asthma. [41] Recent prospective studies reported that 20%-35% of patients with asthma experience asthma attack during exposure to stress. [42] A wide variety of psychosocial stresses, such as negative life events, certain personality types, caregiver stress, low socioeconomic status, and poor family relationships, have a significant impact on both childhood and adult asthma symptoms. [43-47]

Stress affects not only compliance and self-management but also the pathophysiological process of asthma itself. Stress caused by academic examinations promotes eosinophilic inflammation and IL-5 production, leading to asthma exacerbations in children with mild asthma. [48] In an 18-month

prospective study of asthma, exposure of children to an acute negative life event (e.g., death of a close family member) increased the risk of asthma attack in the subsequent 4 weeks by nearly 2-fold (odds ratio: 1.71, 95% CI: 1.04-2.82). When acute life events occurred in the context of high chronic stress, the risk of asthma attack increased almost a 3-fold (odds ratio: 2.98, 95% CI: 1.20-7.38) in the subsequent 2 weeks. [44] Bereavement was associated with a higher risk of asthma hospitalization and a lower use of asthma medication in a nationwide cohort study of all singleton children (*n* = 5,202,576) born in Denmark during 1977-2008 and in Sweden during 1973-2006. [49] Negative affect was found associated with decreases in spirometric lung function (forced expiratory volume in 1 second (FEV1)) and increases in airway inflammation (fractional exhaled nitric oxide (FeNO)) in asthma patients. Both acute and chronic stress are shown to increase airway inflammation and proinflammatory cytokines in the airways such as IL-4. [42, 50, 51] Moreover, psychological stress is found to be associated with asthma that is more difficult to control, [52] with more frequent and longer hospitalisations, [49] with poor compliance to treatment or more psychiatric symptoms, [42] and greater functional impairment. [53]

2.1.3 Psychological stress and asthma onset

The aetiology of asthma involves complex interactions between genetic, environmental and psychosocial factors. Recent studies suggest that stressful life events and perceived stress are associated with asthma onset. [54, 55] The first attack of asthma can be triggered by a stressful life event, such as death of a family member or mourning. Parental report of stress experience is prospectively associated with risk of wheezing among children during the first 2 years of life. [56] In a survey in 4,010 middle-aged respondents, breaking off a life partnership predisposed to a 2.2-times higher risk of incident asthma 10 years later. [57] Goodwin conducted a birth cohort study of over 1,000 young

participants to the age of 21 years and found that childhood adversity exposure accounted for some of the comorbidity of asthma and depressive and anxiety disorders. [58] In a population-based cohort study of 16,881 participants, Lietzen et al. found that stressful life events increased the risk of asthma onset (HR = 1.96, 95% CI: 1.22-3.13). Moreover, this association was independent of demographic characteristics, smoking status, and exposure to allergens. [59] Except for acute stressful experience triggered asthma onset, studies found that chronic stress such as inadequate parental support and chronic caregiver stress were also associated with asthma exacerbation and the development of allergic diseases. [60] In a systematic review and meta-analysis, Chida et al. reported a positive association between chronic psychological stress and the presence of future atopic disorders including asthma. [61] This suggests that stress may play a prominent role in the development and continuation of asthma. [62]

In a 10-year longitudinal study of 5648 individuals without asthma or allergic rhinitis at baseline, [55] perceived stress was associated with a higher risk of self-reported asthma incidence, daily intake of asthma medication, and first-time asthma-caused hospitalization in a dose-dependent manner. This association was independent of participants' sociodemographics, history of parental asthma, smoking status, and lung function at baseline. In summary, chronic stress may accelerate, and acute stress may trigger, the onset of asthma. The impact of stressful life events varies subjectively due to the considerable variability in perceived stress which is determined by people's cognitive assessment, and emotional and physiological responses to stress. [63] It is therefore essential to assess both stressful life events and the inter-individual variance of subjective perception (perceived stress) in the evaluation of the impact of stress on individuals.

2.1.4 Psychological stress and COPD

High levels of stressful life events and perceived stress are reported in patients with COPD. [64] Moreover, psychological stress is a predictor of adverse health effects, a high risk of relapse or readmission after emergency treatment, and high use of community health care resources in COPD. [64-66] Among patients with COPD, an intrinsic source of stress is directly related to their illness, involving the experience of anxiety and stress provoked by breathing difficulties, including muddled thoughts, heightened emotions, extreme fear and panic and decreased physical energy, and various difficulties in emotional functioning, sleep and rest, physical mobility, social interaction, daily activities, recreation, work and finance. [67-69] Emotional arousal, of either positive or negative affect, triggers dyspnea in patients with COPD. [70] Stress level remained high over time in COPD patients even after discharge from hospital, suggesting a persistent high level of stress experienced by patients during both acute and stable phases of the illness. [71, 72] Gueli et al. found that perceived stress contributed to lung inflammation, as manifested by the significantly higher leukotriene B4 (LTB4), IL-8 and tumor necrosis factor alpha (TNF-α) levels, in patients with stable COPD. [73]

2.1.5 Underlying mechanism

Although hypothesized to be a complex neuroendocrine-immune system interaction [74] in which the HPA axis plays a major role, the underlying mechanism of the stress-airway obstruction association is complex and has not been well elucidated.

A hypothesis of stress-induced inflammation was proposed based on study findings that psychological stress exacerbates symptoms of inflammatory disorders such as asthma, rheumatoid

arthritis and inflammatory bowel disease. [43, 75, 76] The inflammatory activity is regulated by the central and peripheral nervous systems, and related neurotransmitters. Stress may activate the HPA axis and the sympathetic nervous system, leading to release of cortisol and catecholamines that influence cell trafficking, proliferation and function, and cytokine and inflammatory mediator production. Neural responses to stress promote inflammation, and increases in inflammation in turn enhance neural sensitivity to stress. This suggests a bidirectional communication between neural systems and inflammatory process in which stress-related neural activity and inflammation may be mutually promoting and, over time, form a recursive loop that increases both levels of inflammation and risk of psychiatric symptoms. [77, 78]

Duration of stress exposure appears to be an important modulator of stress, and different effects of acute versus chronic stress on the immune system have been reported. Acute stress activates the HPA axis, leading to consequent cortisol release and reduction of airway inflammation; while continuous prolonged or intermittent stimulation, as in chronic stress, dampens HPA axis responsiveness and its anti-inflammatory effect. [42, 79] This is corroborated by clinical studies in which asthma patients who are not treated with inhaled corticosteroids (ICS) are likely to experience an attenuated activity and/or responsiveness of the HPA axis. In line with this concept, most asthmatic children demonstrate improved HPA axis responsiveness on conventional doses of ICS, as their airway inflammation subsides. [80, 81]

It is well-known that stress response shows marked inter-individual variability which is likely to be determined by genetic, biological and environmental factors, and as indicated above the timing of

stress exposure. A strong determinant of such inter-individual differences in stress response may be found in neuropeptides released by neurons that act as neuronal signalling molecules and modulate the release of neurotransmitters and hormones. [22]

2.2 Inter-individual variability of stress and asthma

Because asthma often has its origins in early life, childhood asthma has received widely attention in research. However, asthma is a chronic respiratory disease affecting all age groups, though with distinct age characteristics and significant inter-individual variability. Individualized treatment regimens for asthma need to be established according to the characteristics of patients depending on their ages and inter-individual differences.

2.2.1 Age characteristics of asthma

As a leading cause of disease burden, asthma affects individuals across the entire age spectrum from infants to the elderly. With the increase of age, the predominance of boys in childhood among individuals with asthma gradually diminishes until puberty when a higher incidence of new cases of asthma is observed in girls, and adult asthma affects more women than men. [82] Childhood asthma is among the most common chronic paediatric medical disorders which shows relatively high frequencies of EMD visits, prolonged hospitalizations, and low mortality. [83] Moreover, the clinical severity of asthma in childhood is reported to be a risk factor for the persistence and severity of asthma in adulthood. [84] Psychological factors play an important role in the exacerbation and treatment of childhood asthma. Miller et al. [85] reported that children who died from asthma experienced a recent or impending separation or loss and had high levels of hopelessness and despair

in the days preceding their deaths. In a small-scale study, psychological intervention including relaxation, cognitive stress management, and a self-esteem workshop improved FEV1 and decreased specific immunoglobulin E (IgE) response in asthmatic children. [86] However, young children may have difficulty in reporting their psychological status accurately and consistently. Parental assistance generally exaggerates the physical and psychological symptoms children experience and causes potential bias. [87] Therefore, young children are often excluded from psychological studies which rely on self-reports of emotions.

Adolescents are in a period of remarkable physical, psychological and personality development, which is filled with rapid changes, roles adaptation and feelings of self-doubt. Hence, they are vulnerable to the negative impact of asthma. [88] Adolescent asthma is relatively dynamic with 1 in 5 young children with asthma remit but may subsequently relapse after a symptom-free interval. [89] Patients in this age group usually underestimate the severity of their disease and denial of symptoms is characteristic of adolescents with asthma. They are usually not willing to take asthma medication in front of their peers and have poor adherence to prescribed treatment regimens. The under-estimation and inadequate treatment of asthma symptoms often induce poor outcome of asthma in adolescence. Studies showed that it is more common among adolescents than younger children to experience asthma exacerbations requiring hospitalization, intubation, and cardiopulmonary resuscitation and fatal outcomes. [90] In a study conducted in Dutch, 27% of adolescents with asthma believed that their asthma symptoms were provoked by prolonged emotional arousal or stress, and 40% of the participants linked their symptoms to sudden anger or sadness. [91] Compared with their healthy counterparts, adolescent asthma patients exhibit higher levels of anxiety and depression, increased suicidal ideation and lower self-esteem. [92, 93] The severity of psychiatric comorbidity is

correlated to the severity of asthma symptoms, impaired functioning and poor adherence to treatment regimens. [94, 95]

Adult asthma, with approximately 60% persisting from childhood and 40% adult-onset, is relatively stable compared with other age groups. [96, 97] Adult-onset asthma is mainly non-atopic and affects predominately females. It is frequently associated with a variety of specific triggers such as smoking, respiratory tract infections, aspirin intake, exposure to occupational agents, and obesity. Most patients with adult-onset asthma have mild transient disease, whereas others exhibit a progressive course with high exacerbation rate and rapid loss of lung function. [98, 99] The increased prevalence of asthma morbidity and mortality has been linked to excessive stress and emotional strains in modern life. [100] Levitan reported six adults whose symptoms of asthma began during periods of intense mourning. [101] Among them, four patients reportedly manifested asthma 1 week to 3 months after the death of a relative, and the other two patients on the day of the death or funeral. Rumbak et al. found that a majority of the subjects with asthma responded the "frequently" or "always" category when asked how often asthma attacks were preceded by their being upset or anxious. [102] Watching stressful films increase airway obstruction in adults with mild asthma. [103] In population-based studies, stressful life events were associated with an increased risk of hospital admissions due to asthma. [104] There may be a vicious circle of bidirectional interaction between asthma and stress which affects the management of asthma and patients' quality of life.

In the elderly, the diagnosis of asthma is often difficult due to greater irreversibility of airflow obstruction, comorbidities with COPD and other chronic medical conditions, and the advancement of

age, leading to under-diagnosis and under-treatment of asthma in the elderly population. [105, 106] Compared with younger age groups, older individuals with asthma tend to have lower incomes, less formal education, higher requirement for systemic corticosteroid therapy, shorter symptom-free periods, and more daily activity limitation because of asthma. [107-109] While higher asthma prevalence is observed among younger age groups, elderly individuals with asthma have worse control of asthma and a higher mortality than other age groups. [110] In the United States, over 50% of asthma-related deaths annually occur in asthma patients aged 65 years and above. [111] Although asthma is not considered a common direct cause of death among the elderly, the co-existence of asthma and other risk factors, such as smoking, cardiovascular disease, and psychological symptoms, may exert a heavy burden on health care utilization and partially account for the excess fatalities. [111, 112] Consistent with the clinical criteria of diagnosing airflow obstruction, [113] this study employed an objective parameter of chronic airflow obstruction defined as post-bronchodilator FEV1/forced vital capacity (FVC) < 0.70 in order to avoid the confounding of the non-specific symptoms and signs of airway disease in the elderly. Important demographic and disease-related variables including sex, age, ethnicity, smoking status, and number of medical comorbidities were adjusted in the statistical analyses in the elderly population.

2.2.2 Inter-individual variability of stress

The relationship between stress exposure and psychopathology is extremely complicated. It can be illustrated by considering not only stress exposure but also the vulnerability and resilience of the individual who is exposed to stressors. It is well-known that there is marked inter-individual variability in responses to stress. Some individuals are more vulnerable, while others are more resilient. Many individuals who are exposed to similar levels of adversity with those having PTSD do

not develop psychopathology or are able to recover quickly. This is determined by a variety of genetic, neurobiological, environmental, and psychological factors. [114]

The neurobiological responses to stress involve the central nervous system, peripheral nervous system, related neural circuits and neurotransmitters, neuropeptides, and hormones, in particular the HPA axis. [115] The central control stations of stress response are located in the hypothalamus and the brain stem. When confronted with a stressful situation, activation of the HPA axis initiates the production of corticotropin-releasing hormone (CRH), stimulates the secretion of ACTH from the anterior pituitary, and finally activates the secretion of corticoids by the adrenal cortex. In the meanwhile, the coeruleus activates the sympathetic nervous system, and releases adrenaline and noradrenaline at the sympathetic nerve endings. The released catecholamines and corticoids suppress Th1 cell response such as the production of IL-12 and promote the Th2 cell production such as IL-4, IL-10 and IL-13. This results in a Th1/Th2 imbalance in favor of Th2 cell mediated response, with dysregulation of the neuroimmunologic homeostatic mechanisms secondary to chronic stress, which ultimately affects cytokine expression and favours an allergic inflammatory response such as asthma. [116]

There are a number of key neuroendocrine mediators involved in the neurochemical response patterns to stress, such as cortisol, CRH, ACTH, noradrenaline, and so on. Evidence has demonstrated that psychological stress increases the synthesis and release of cortisol which replenishes energy stores, contributes to increased vigilance, and inhibits the growth and reproductive system and the immune response. [117] CRH coordinates stress-related adaptive

behavioural and physiological changes. During stress, release of CRH activates the responsiveness of the HPA axis and increases cortisol and dehydroepiandrosterone (DHEA) secretion. The activity of the CRH neurons is associated with the activation of fear-related behaviours, reduction of reward expectation, and inhibition of a variety of neurovegetative functions such as food intake, sexual activity, and endocrine programs for growth and reproduction. [118] ACTH, secreted by the anterior lobe of the pituitary gland into the body's blood stream, stimulates the cortex of the adrenal gland by binding to its ACTH-receptors and promotes the release of cortisol from the adrenal gland. As a stress hormone, noradrenaline affects brain regions that are responsible for attention and responding to actions. Noradrenaline and adrenaline form the basis for the fight-or-flight response by directly increasing heart rate, stimulating glucose release from stored energy, and enhancing blood supply to skeletal muscles. [23]

In 1982, Tatemoto and Mutt [119] extracted NPY from porcine brain. Accumulating evidence suggests that, under physiological conditions, NPY functions as an endogenous anxiolytic agent that buffers against the effects of stress on the mammalian brain. [120] The role of NPY in stress resilience as a major neurotransmitter remains to be fully elucidated.

2.3 Neuropeptide Y and inter-individual variability in stress-asthma link

NPY is a peptide which has widespread central and peripheral distribution and multiplephysiological effects. As a neuropeptide, the anxiolytic effect of NPY via the amygdala has received growing attention. Psycho-neuro-immunological studies on the role of NPY as a resilience factor in

interpreting the inter-individual variability of stress help to illuminate neurotransmitter imbalances and dysfunctions involved in response to psychological stress. [121]

2.3.1 Structure and function of Neuropeptide Y

NPY is a highly conserved 36-amino acid peptide neurotransmitter found in the arcuate nucleus of the hypothalamus. It is one of the most abundant peptides in the central nervous system of mammals and regulates multiple physiological activities. [122] In the endoplasmic reticulum, NPY is synthesized as a large precursor protein. Once synthesized, NPY moves to the Golgi apparatus, and is then translocated into the trans-Golgi network (TGN) where the peptide is stored until further activation. [123] NPY is most abundant in the neurons of five brain areas including the locus coeruleus, the paraventricular nucleus of the hypothalamus, septohippocampal neurons, the nucleus of the solitary tract, and the ventral lateral medulla. Moderate levels of NPY are found in the amygdala, hippocampus, cerebral cortex, basal ganglia, and thalamus. [124] At these brain areas, NPY is shown to have two major functions: stimulating food intake and modulating stress response. Injection of NPY into the paraventricular nucleus stimulates strong feeding-stimulatory responses; [125] while injection of NPY into the paraventricular nucleus triggers the secretion of ACTH which initiates a cascade of stress-related events and results in the release of cortisol. [126]

In the periphery, NPY is mainly found in sympathetic nerves, the adrenal medulla, and platelets. Studies have shown the co-release of NPY, noradrenaline, and Adenosine Triphosphate (ATP) from axon terminals under isolated, in situ and in vivo stimulation of organs by sympathetic activation. [127,

[128] Clinically, elevated plasma NPY levels are observed in diseases with increased sympathetic outflow such as hypertension, [129] chronic heart failure, [130] and renal failure. [131]

In animals, NPY is found to subserve functions in regulating behaviour and adaptation of the organism during environmental challenges such as starvation, infection and predator attack. [132, 133] Human studies found that NPY is involved in the regulation of several basic physiological functions and disorders such as the control of anxiety, seizures, memory, circadian rhythm, food intake, metabolic disorders, drug addiction, pain, cardiovascular diseases, rhinitis, and endothelial cell dysfunctions, through the activation of six G-protein-coupled receptor subtypes named Y1, Y2, Y3, Y4, Y5, and Y6. [123] NPY co-exists with stress hormones adrenaline and noradrenaline in neurons of the nucleus of the solitary tract and the autonomic nervous system, augments the vasoconstrictor effects of noradrenergic neurons, and regulates the release of many neurotransmitters including adrenaline and noradrenaline.

2.3.2 Neuropeptide Y and psychological stress

There is interplay between the NPY system and the classical HPA axis and the sympathetic-adrenomedullary (SAM) axis stress response systems both centrally and peripherally. Exposure to an acute stress leads to the expression and release of NPY in multiple stress-sensitive areas of the brain. [134] The negative feedbacks of NPY-ergic activity, which is in anxiolytic fashion on CRH, may be regarded as “anti-stress”. [120] On the other hand, glucocorticoids are capable of modulating the expression of NPY messenger ribonucleic acid (mRNA) in the arcuate nucleus, thus exacerbating the

response to stress. [135] Meanwhile, similar interactions between glucocorticoids and NPY are also observed in the periphery. [136]

High NPY levels are associated with better performance in soldiers of special operations under acute and extreme training stress, but chronic stress seen in long-term patients with PTSD is shown to reduce plasma NPY levels. [137, 138] Low levels of NPY are also found in depressed patients, and a variety of antidepressant drugs increase NPY levels. The balance between NPY and CRH neurotransmission is therefore important to the long term emotional responses to stress and reduction of the allostatic load. Although evidence is not extensive, these studies indicate the possibility of NPY playing a key role in triggering and propagating the stress response.

2.3.3 Neuropeptide Y and inter-individual variability of stress in asthma

Evolutionarily, the function of NPY appears to be under conditions of cold climate and in situations where food is sparse, as the peptide stimulates coping and food-seeking behaviour, and regulates cardiovascular, neuro-endocrine and immune adaptations. [135] Under stress, NPY exerts a major influence on humoral and cellular immune functions, which are intimately involved in asthma pathophysiology. NPY modulates potent immunological effects such as immune cell distribution, T helper cell differentiation, mediator release, and natural killer cell activation. [139]

NPY exists not only in neurons, but as well in neuroendocrine and inflammatory cells in the lung. Exposure to stressful life events may result in the release of NPY in the airways and peripheral

circulation during asthma exacerbations. [139] There are a few studies investigating the role of NPY in asthma in prior research. Dahlof et al. found elevated plasma concentration of NPY and low level of circulating adrenaline in elderly asthmatics during rest and acute severe asthma compared with the healthy controls. Cardell et al. reported that during an exacerbation of asthma, patients displayed low plasma concentrations of NPY and elevated levels of other neuropeptides. Doniec et al. examined the serum level of NPY (and leptin) in children with mild asthma and observed no difference of NPY levels between the asthmatics and the healthy children. [140-142] Macia et al. in 2011 found that NPY exacerbates allergic airway inflammation (AAI) in mice via its Y1 receptor which is closely related to stress. [143] Aldrich et al. reported that Puerto Ricans exhibit a significant association between single nucleotide polymorphism (SNP) rs5574 and asthma prevalence (OR = 1.28, 95% CI: 1.02-1.60). Two SNPs, rs16143 and rs5574, are associated with severe asthma (OR = 1.48, 95% CI: 1.11-1.96 and OR = 1.44, 95% CI: 1.09-1.89, respectively). However, among Mexicans, associations of asthma and NPY variants are not statistically significant. [144] The association of stress and asthma suggested by these preliminary studies deserves further investigation.

CHAPTER 3 METHODS

3.1 Study I: Prevalence of anxiety and depressive symptoms in adolescents with asthma: a meta-analysis and meta-regression

3.1.1 Search strategy

An extensive literature search was performed using the keywords "depress*", "anxiety", "psych*", "behaviour", "paediatric", "children", "adolescent", "youth", and "asthma", to retrieve case-control studies published in multiple computerized databases: PubMed (from 1966 to Nov 2011), Embase (from 1980 to Nov 2011), BIOSIS (from 1926 to Nov 2011), PsychINFO (from 1806 to Nov 2011), Science Direct (from 2006 to Nov 2011) and Cochrane CENTRAL (from 1993 to Nov 2011). Relevant articles were also searched from the bibliographies of the retrieved original studies and review articles. We contacted the corresponding authors for any information lacking in their published articles to calculate the effect size.

3.1.2 Criteria for article selection

Observational case-control studies addressing the difference in depressive and/or anxiety symptoms between adolescents with asthma and the healthy controls were included in this meta-analysis if they met the following criteria: 1) conduction of human studies involving adolescents (aged 13-18 years) grouped by asthma and the healthy controls; 2) identification of clinically relevant depressive and/or anxiety symptoms; 3) written in English; 4) having a total sample size of no less than 50 subjects. We included both cross-sectional and the first round data collection of prospective studies. Two investigators (YL and RCMH) independently assessed the relevancy of the manuscripts and ineligible manuscripts were excluded according to the following exclusion criteria: 1) comparisons

between asthma patients and the healthy controls were not available; (2) a standardized effect size could not be calculated. Approval from an institutional review board was not required because this meta-analysis mainly involved data from published studies.

3.1.3 Data abstraction

All articles were first de-identified (blinded title, author(s), journal name and year of publication) before selection. Two investigators (YL and RCMH) independently reviewed the abstracts of the studies and extracted relevant data from eligible papers into standard electronic forms. Any discrepancies were resolved by consensus. If consensus could not be reached, the principal investigators (RCMH) would make the final decision for study eligibility and data extraction.

3.1.4 Statistical analyses

All statistical analyses in this meta-analysis were performed using the Comprehensive Meta-analysis Programme, Version 2 (Biostat, Englewood, NJ, USA). Random effects meta-analysis was performed as suggested by DerSimonian and Laird [145] to test whether the aggregate prevalence of depressive and anxiety symptoms was the same in these two groups. The forest plots were prepared using R. [146]

As the number of studies included in this meta-analysis is relatively small, the Cochran Q-test for heterogeneity may yield a low statistical power. [147] A value of significance at 10% ($p \leq 0.1$) was therefore considered statistically significant for heterogeneity. [148] Meanwhile heterogeneity was

assessed with I^2, which is defined as the percentage of total variation of all studies caused by heterogeneity rather than change. High values of I^2 suggest increased heterogeneity. Publication bias was evaluated with Egger's test in our analysis.

For models with considerable heterogeneity and statistically significant effect size, meta-regression was performed to identify demographic and disease-related factors (age, gender, ethnicity, smoking status and severity of asthma) that might contribute to the heterogeneity. [149] Since the chosen covariates were not expected to explain all the heterogeneity of the studies, mixed-model meta-regression was used in consideration of the presence of "residual heterogeneity". [149] The regression coefficients and the associated standard error (SE), the z score, degree of freedom (df), and p values were reported for the meta-regression analysis.

3.2 Study II: Asthma control, perceived stress, and quality of life in adolescents with asthma: a case-control study

3.2.1 Study design and participants

This is a clinical study with a cross-sectional design. Adolescents (aged 12-19 years) with asthma diagnosed by a paediatrician (HPVB) were recruited from April 2011 to June 2012, including 137 patients with well-controlled asthma and 61 with poorly controlled asthma from the specialist clinics and wards of Department of Paediatrics, National University Hospital (NUH). A group of 171 healthy controls were neighbourhood volunteers age-matched (+/-2 years) and gender-matched with asthma patients, without current or previous history of asthma. Adolescents with other chronic diseases, especially any other pulmonary disease (cystic fibrosis, etc.), mental incapacitation or

learning disability, with difficulty understanding and responding to questions were not eligible for this study. All participants and their parents signed written informed consent for the study which was approved by the National Healthcare Group Domain Specific Review Board (DSRB). The response rate was 92%. Self-administered questionnaires were completed in English by the respondents who were multi-ethnic but received a common education in English as first language.

3.2.2 Asthma control and asthma quality of life

The degree of asthma control was determined using the Asthma Control Test (ACT), a widely-used self-administered questionnaire in clinical and research settings for the assessment of asthma with strong evaluative and discriminative properties in general [150] and locally [151]. Participants responded to 5 questions pertaining to episodes of dyspnea, sleep impairment due to asthma symptoms, limitations in daily activities, the use of rescue inhalatory medications, and self-rated degree of asthma control in the past four weeks. Participants responded to a 5-point Likert scale. A score of 20 points or higher corresponded to well-controlled asthma and equal to or less than 19 suggested poorly controlled asthma.

The impact of asthma on children's quality of life was assessed by the Paediatric Asthma Quality of Life Questionnaire (PAQLQ), which has been extensively tested for reliability and validity in multiple populations [152] including Singaporeans [153]. The questionnaire includes 23 items in 3 domains: activity limitations (5 items), symptoms (10 items), and emotional function (8 items). Questions are regarding how bothered in the past week children were by cough, wheeze, tightness of the chest and shortness of breath, and asthma attacks, limitation with physical activities, exposure to

animals, and activities with friends and family, etc.. Response options for the questions were 7-point Likert scales ranging from “extremely bothered” to “not bothered” or “all of the time” to “none of the time”. A lower PAQLQ score indicates impaired quality of life.

3.2.3 Depression and anxiety

Revised Child Anxiety and Depression Scale (RCADS) is a 47-item self-administered questionnaire for screening a broad range of *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-Ⅳ)* anxiety and depressive disorder symptoms in adolescents and young children, [154] with scales corresponding to panic disorder, social phobia, generalized anxiety disorder (GAD), obsessive compulsive disorder, separation anxiety disorder, and major depressive disorder (MDD). The questionnaire also derives scores on Total Anxiety Scale (sum of the 5 anxiety subscales) and Total Internalizing Scale (sum of all the 6 subscales). Respondents are required to rate how often each item applies to them on 4-point Likert scales with the options of “never,” “sometimes,” “often,” and “always”. Raw scores are transformed into standard T scores based on subjects’ gender and grade levels. T scores of 65 or higher indicate that the subject is at the borderline clinical threshold of anxiety or depressive disorders. T scores of 70 or higher are above the clinical threshold of psychiatric morbidity. RCADS has shown excellent psychometric properties and robust construct validity [154] in cross-validation studies including in Asian countries and is available in multiple languages. [154]

3.2.4 Stressful life events and perceived stress

The Holmes-Rahe Social Readjustment Ratings Scale [155] (SRRS, for children) is used to measure the number of significant stressful life events experienced by participants in the past one year. The scale examines stressful life events that necessitated life-style changes requiring social readjustment. Stressful life events listed on the SRRS pertain to significant areas in the social structure of daily life, including marriage, pregnancy, education, occupation, family, group and peer relationships, economic concerns, religion, recreation, and health. This scale has been validated cross-culturally for Asian populations. [156, 157]

Perceived Stress Scale (PSS) is a widely used psychological instrument for the measurement of perception of stress and has been validated in many studies [158] and in Singaporean samples [159]. It measures the degree to which situations in one's life is appraised as stressful. Items are designed to detect how unpredictable, uncontrollable, and overloaded respondents find their lives over the past month. All items begin with the phrase: In the past month, how often have you felt…? The questions are of a general nature and hence are not directed at any particular sub-population group. The scale derives a perceived stress score that ranged from 0 to 40 with a higher score indicating experience of greater stress. High PSS scores were shown to be associated with poor self-reported health status and vulnerability to depressive symptoms. [160]

3.2.5 Socio-demographic data and self-rated health

We measured socio-demographic variables such as gender, age, ethnicity (Chinese, Malay, Indian or others), smoking status (non-smoker, past or current smoker), family housing type as a proxy for socioeconomic status (1-2 room public, 3 room public, 4-5 room public, private or landed house),

and Body Mass Index (BMI).

Self-rated health (SRH) was assessed with one item "Would you say that in general your health is…?" Participants were asked to answer with a 5-category Likert response scale (excellent, very good, good, fair or poor). Self-rated health is a widely used measure of general health and has been shown to predict subsequent mortality and morbidity in both population and clinical samples, even after accounting for socio-demographic and other health-related characteristics. [161, 162]

3.2.6 Statistical analyses

Data analysis was performed using Predictive Analytics Software (PASW) Statistics version 18. Differences in socio-demographic status, measures of psychiatric comorbidity, underlying psychological factors and their correlates, and QoL were investigated among groups with poorly controlled asthma, well controlled asthma versus the healthy controls, using 2-tailed Chi-squared test and One-way Analyses of Variance (ANOVA), with pairwise comparisons between groups using Bonferroni adjusted p values. Pearson correlational analysis was used to examine the relationship between asthma, psychological and psychiatric variables. The scores of psychiatric comorbidity were compared among the three groups in generalized linear regression models, controlling for potential confounders (gender, age, ethnicity, smoking status and family housing type). To evaluate the role of perceived stress in mediating the observed difference in psychiatric comorbidities among the participants, we included the variable in the prediction model of psychiatric comorbidities, together with diagnostic checks for multi-collinearity. The change in the strength of association, the level of statistical significance and R-squared value in various regression models were assessed.

3.3 Study III: Asthma, psychological stress and psychiatric comorbidity: a population-based study in adult Singaporeans

3.3.1 Study design and participants

This study used cross-sectional data from a national stratified random population sample (n = 2847) in the National Mental Health Survey (Adults) (NMHS (A)) of Singapore, conducted from 15 February 2003 to 30 March 2004. The random sample list was generated by the Department of Statistics using pure probability sampling from a national sampling frame based on population census of persons residing in coded districts throughout the country. The NMHS (A) used an ethnically stratified random sample of households to identify eligible adults aged 20-59 years who were Singapore citizens or permanent residents. One eligible person per household was invited to participate in the survey. A total of 2,847 individuals were interviewed from 3,875 randomly selected eligible persons, with a response rate of 75.2%. A multi-language team of trained field interviewers performed face-to-face questionnaire interviews at the subjects' home. The interviews were conducted in the language or dialect with which the subjects were most conversant: English, Mandarin or other Chinese dialects, and Malay. Participants gave written informed consent for the study which was approved by the Ethics Committee of the Institute of Mental Health.

3.3.2 Asthma and other chronic physical conditions

The presence of asthma was ascertained by subjects' self-reports of a doctor's diagnosis of asthma: "In the past 12 months, have you been told by a doctor, nurse, or other health professional that you had asthma?" ("yes", "no"), and "Do you still have asthma?" ("yes", "no"). Self-reports of asthma

diagnosis based on these two questions are shown high reliability and validity in multiple population surveys of asthma prevalence. [163-167] Additionally, the respondents were asked to show their medication packages, and were considered to have asthma when the use of appropriate asthma medications was verified by the interviewer. Additional questions for those with a positive response of asthma diagnosis included their duration of asthma, frequency of doctor visits and hospitalizations for asthma exacerbations.

In the meanwhile, the subjects were asked whether in the 12 months prior to interview they had been diagnosed and treated by their doctors for any of 14 other chronic physical illnesses ("yes", "no"), which included coronary heart disease, heart failure, hypertension, lipid abnormalities, diabetes, stroke, cancer, hip fracture, arthritis, chronic obstructive pulmonary disease, cataract, kidney failure, urinary disorders, and "other specified conditions". The self-reported diagnoses of physical illnesses were corroborated by information provided on the use of appropriate medications as verified by the interviewer, and duration of illnesses, frequencies of doctor visits and hospitalization in the last 12 months. Two respondents who reported both chronic obstructive pulmonary disease and asthma were classified among the 144 respondents who had asthma. The number of chronic physical illnesses was calculated for each individual, and the sum score of number of coexisting physical illnesses except the index illness was used as a covariate in the statistical analysis.

Based on information on physical medical conditions provided, individuals were categorized in three groups of study participants: asthma (A), other chronic physical conditions (B) and no chronic physical conditions (C). Thirty-eight individuals having both asthma and other chronic physical

conditions were excluded from the asthma group and in the analysis.

3.3.3 Psychiatric disorders

Diagnosis of psychiatric disorders was made using a two-stage case ascertainment procedure, in which participants were first screened using the General Health Questionnaire (GHQ)-12, and screen-positive individuals (GHQ $\geq$ 2) underwent diagnostic interviews for psychiatric disorders including MDD, GAD and others using the Schedule for Clinical Assessment in Neuropsychiatry (SCAN).

The GHQ-12 [168] is widely used by researchers and clinicians as a screening instrument to detect psychiatric disorders in community settings and non-psychiatric clinical settings. [168] The questionnaire has demonstrated stable discriminating power cross-culturally [169] in 11 translated versions, and its validity has been documented in previous studies in Singapore. [170, 171] A recommended cutoff score of 2 [172, 173] denotes psychological disturbance. Among respondents with GHQ scores of at least 2 who underwent further diagnostic interview using the SCAN, [174] the positive predictive value was 31%. In validation studies, a random one-in-ten of the subjects with GHQ-12 scores below 2 were also further interviewed using the SCAN to address false-negative results from screen-negative respondents, and the GHQ-12 gave a sensitivity of 92.1% and specificity of 48.1% for depression/anxiety. [175]

Interview data generated from SCAN (created by World Health Organization) were used to make

case diagnoses of psychiatric disorders including MDD, GAD and others in the previous 12 months period in accordance with *DSM-IV* [176] criteria. SCAN has been found to be generally acceptable, appropriate and reliable across cultures and settings [177] when administered by trained lay interviewers in community surveys. [178, 179] Case diagnoses of psychiatric disorders including any psychiatric disorder, MDD and GAD were evaluated as dichotomous variables (0 = no, 1 = yes) in the statistical analysis. The multi-ethnic interviewers were experienced psychiatric nurses from the Institute of Mental Health who were trained in the use of the SCAN by a senior psychiatrist who had received training from a WHO training centre. To ensure the conduct of interviews in accordance with the study protocol and accuracy of diagnoses, quality checks was conducted through weekly on-site supervision and cross-checking of interview response data by the research coordinator and psychiatrists.

3.3.4 Stressful life events and quality of life

Subjects were asked whether they had experienced any stressful life events in the previous 6 months from a List of Threatening Experiences Questionnaire (LTEQ). [180] The LTEQ consists of 12 items of stressful life events, including serious illness or injury to self or close relative, death of parent or spouse or child, etc.. A total number of reported stressful life events, as well as in each type (threat, loss or mixed) was computed for each subject. Missing values for items were given null values for a small number (< 5%) of items and study participants.

Health-related quality of life was measured using the Medical Outcomes Study 12-item Short Form (SF-12) which has been widely used in clinical and large population health surveys including

samples of Singaporean adults. [181-183] Scores are computed on two scales of mental and physical functioning, Physical Component Summary (PCS) and Mental Component Summary (MCS), using the weighted scores of all twelve questions. The possible scores range from 0 to 100, where zero indicates the lowest level of health functioning measured by the scales and 100 indicates the highest level of health functioning.

3.3.5 Statistical analyses

Differences in socio-demographic factors, any psychiatric disorder, MDD and GAD, and number and type of stressful life events were evaluated among the three study groups using 2-tailed Chi-squared test. Crude and adjusted odds ratio (OR) of association of psychiatric disorders (any psychiatric disorder, MDD and GAD) with asthma versus no chronic physical conditions (A versus C), other chronic physical conditions versus no chronic physical conditions (B versus C), and asthma versus other chronic physical conditions (A versus B) were estimated in univariate and multivariable logistic regressions. To control for potential confounding, the latter included as covariates sex, age, ethnicity, employment status, marital status, highest education, and number of coexisting chronic illness, determined a priori to be known important risk factors for psychiatric disorders or morbidity.

To measure the role of stressful life events in mediating the association of coexisting psychiatric disorders with asthma and other chronic physical conditions, the author evaluated the change in the magnitude of ORs in hierarchical models by including number of stressful life events in the model and observing the decrease (to non-significant value or zero) in the OR of association between asthma (or other chronic medical conditions) and psychiatric disorder. [184] Sobel test was used to

evaluate the statistical significance of the mediation. Reported p-values were obtained from the unit normal distribution under the assumption of a two-tailed test.

The mean level of SF-12 MCS measure of QoL was compared among the groups with asthma, other chronic physical conditions and without chronic physical conditions. Potential confounders were adjusted in analyses of covariance using generalized linear regression models. We added separately in the model number of stressful life events, any psychiatric disorder, MDD and GAD in order to compare their relative contributions in explaining the observed differences in QoL.

3.4 Study IV: The impact of stressful life events on quality of life in the elderly with airway obstruction

3.4.1 Study design and participants

The participants in the study were a subsample (n = 497) recruited from one locality (Bukit Merah) in the South Central region of Singapore Longitudinal Ageing Study (SLAS)-2 which is a prospective population-based cohort study of aging and health of community-dwelling elderly. [185] Trained research nurses interviewed one participant from each household who were Singaporean citizens or permanent residents aged 65 or older and were able to give informed consent. Those who were too frail or ill and unable to complete the interview, for reasons such as from post-stroke aphasia, cachexia or profound dementia, were excluded. All participants signed written informed consent for the study which was approved by the DSRB.

3.4.2 Airway obstruction

Ventilatory function testing was performed using a portable, battery operated, ultrasound transit-time based spirometer (Easy-One; Model 2001 Diagnostic Spirometer, NDD Medical Technologies, Zurich, Switzerland). Calibration was checked daily with a 3-L syringe. Forced expiratory maneuvers were performed with the respondent seated according to American Thoracic Society (ATS) recommended guidelines and standardization of procedures: at least three acceptable maneuvers, with FVC and FEV1 reproducible within 200 ml. Satisfactory spirometric tests were performed in 83.1% of the respondents. The respondent was given 1 puff (100 µg/puff) of inhaled ventolin immediately after pre-bronchodilator maneuvers, followed by 2 puffs of inhaled ventolin 5 min later. Chronic airflow obstruction was defined as Post-bronchodilator FEV1/FVC < 0.70, consistent with the clinical criteria of diagnosing airflow obstruction. [186]

3.4.3 Stressful life events and depressive symptoms

Stressful life events were measured by the 11-item life events inventory [71, 187] that excluded personal illness experience directly related to airflow obstruction. The participants were asked to indicate yes or no as to whether any of eleven stressful life events had occurred over the past year ("spouse or partner die, a close friend or family member (other than spouse or partner) die or have a serious illness, major problems with money, a divorce or breakup, family member or close friend have a divorce or breakup, major conflict with children or grandchildren, major accidents, disasters, muggings, unwanted sexual experiences, robberies, or similar events, a family member or close friend lose job or retire, physically abused, verbally abused, or pet die"). If the participant indicated (a) life event(s) had occurred, he/she was asked to appraise the event and indicate on a scale of 1 (did

not upset me) to 3 (upset me greatly) the extent it upset them. The frequency of stressful life events was calculated for each respondent. The scale also provides a stressful life event score appraised by the participant that ranged from 0 to 33 with a higher score indicating a participant experienced a greater number of more stressful life events.

The presence of depressive symptoms was determined by a depression screening scale for elderly populations, the 15-item Geriatric Depression Scale (GDS-15) with scores ranging from 0 to 15. [188] The GDS was well suited for the study because it is largely free of the measurement artefact due to overlapping somatic symptoms of physical illness(es) and depression. Depressive symptoms defined by GDS $\geq$ 5 is clinically significant, and such cases including "sub-threshold" depression, had been shown in the same population to be associated with significantly poorer mental and physical health and functional status, and more healthcare resource utilization compared to non-cases and were similar to or worse than syndrome threshold cases of depression. [189]

3.4.4 Cognitive function

Cognitive function was measured using the Cognitive Failures Questionnaire (CFQ) [190] and the Mini-Mental State Examination (MMSE) [191] which were validated and widely used to assess global cognitive functioning. The CFQ uses a 5-point Likert-type scale (1 = Never, 5 = Very often) to evaluate self-reported cognitive problems (e.g.,''Do you need to re-read instructions several times?"). Higher CFQ scores indicate more frequent cognitive problems. Higher MMSE scores (0 to 30) indicate better global cognitive functioning, and MMSE scores of 23 or less are considered to be cognitively impaired.

3.4.5 Physical and mental functioning

Physical and mental functional well-being was measured by the Medical Outcomes Study 36-item Short Form (SF-36) [192] which has been previously validated for use in Singaporeans [193]. Weighted summary measures of PCS and MCS scores were computed with higher scores indicating better physical and mental health functioning and QoL.

3.4.6 Statistical analyses

In preliminary univariate analysis, participants with and without airway obstruction were compared with respect to differences in number of stressful life events, perceived stress score, level of FEV1, CFQ, MMSE, GDS depression, and SF-36 PCS and MCS scores, as well as potential confounding variables, sex, age, ethnicity, smoking status, number of chronic diseases, using t-tests or chi-squared tests of significance. The independent main effects of stressful life events and airway obstruction (independent variables) as well as the interaction of stressful life events and airway obstruction on measures of pulmonary function, depressive symptoms, cognitive function, and QoL (dependent variables) were analyzed using two-way ANOVA using generalized linear model which adjusted for sex, age, ethnicity, smoking status, and number of chronic illness. The independent variable of primary interest was stressful life events, and the primary outcome variables of interest were depressive symptoms and QoL. A secondary relationship analyzed in the two-way ANOVA model was the main effect of airway obstruction status (and its interaction with stressful life events) on primary outcomes of pulmonary and cognitive functions. For the outcome variables with significant interaction of stressful life events and airway obstruction, the simple effects of their relationships

with stressful life event score were investigated respectively in participants with and without airway obstruction.

3.5 Study V: Stress, neuropeptide Y, and young adult asthma: a follow-up study

3.5.1 Study design and participants

In this clinical study, 70 young adults (aged 21-35 years) with physician-diagnosed asthma were recruited. They included 19 chronic asthma patients with acute exacerbations from EMD, 51 patients with stable chronic asthma on follow up in the clinics of the Division of Respiratory and Critical Care Medicine (RCCM) of NUH. A group of 69 healthy controls were age-matched (+/- 2 years) and gender-matched with asthma patients, without current or previous history of asthma and agreed to provide blood samples, health information and written informed consent.

The inclusion criteria were: asthma patients a) with doctor-diagnosed asthma; b) present at EMD for acute exacerbations of asthma or on routine follow up at RCCM specialist clinics with known asthma in quiescent state (free of asthma attacks) for at least 3 months; c) aged 21-35 years; d) able to understand and respond to questions in English; healthy controls a) aged 21-35 years; b) able to understand and respond to questions in English. The exclusion criteria were individuals a) with other pulmonary disease (cystic fibrosis, etc.); b) having neurological or psychiatric illness (depression, schizophrenia, bipolar disorder, PTSD, ADHD, autistic disorder, etc.); c) having gastro-intestinal (e.g. irritable bowel syndromes), autoimmune disorders (arthritis, lupus, etc.), pituitary syndromes, Cushings syndrome, involving disturbances in the HPA axis; d) on oral steroid treatment within two

weeks before visiting; e) having mental incapacitation or learning disability with difficulty understanding and responding to questions in English.

Interested volunteer patients and healthy controls were invited to attend a screening interview to assess their eligibility and provide informed consent before enrolment. After this, trained nurses would bring the participant to a designated room within the department that is free from distraction and where the conversation between the investigator and the participant could not be overheard. The participants' demographics, asthma clinical, psychological, neuroendocrine and immunological profiles were assessed. Spirometric tests were administered and 20ml venous blood samples were collected into the BD Vacutainer® CPT™ Cell Preparation tube with sodium citrate. Samples were centrifuged at room temperature in a horizontal rotor for 20 minutes at 1650 rpm with the brake off. Within 2 hours after sample collection, plasma was isolated from each blood sample and stored in a −80°C freezer until the time the experiments were performed. At 12-month follow up, 63 asthma patients were successfully re-contacted. All participants signed written informed consent for the study, which was approved by the National Healthcare Group DSRB.

3.5.2 Spirometric test

Lung function testing was performed as described in 3.4.2.

3.5.3 Questionnaire administration

Asthma control was measured using the ACT as described in 3.2.2. Asthma-related functional impairment was assessed using the Asthma Quality of Life Questionnaire (AQLQ) [194, 195] which is a validated questionnaire with excellent psychometric properties: the internal consistency reliability ranged from 0.90 to 0.95, and the reproducibility ranged from 0.81 to 0.93. [196] It consists of 32 items relating to day-to-day problems most troublesome to asthma patients. Consequences of asthma are evaluated in four life domains: activity limitations (ability to carry out daily activities), symptoms (frequency and nature of asthma symptoms such as coughing and chest tightness), emotional distress (intensity of the discomfort, fear, or distress associated with asthma), and exposure (to environmental stimuli) (capacity to manage environmental triggers of asthma such as smoke, dust, and pollution). Patients were asked to recall their experience over the previous 2 weeks and respond to each question on a 7-point scale. Average scores on each domain and a total AQLQ score were generated for each patient.

The SRRS [155] was used to measure stress from major life events. The SRRS has been used widely in studies of psychosocial stress and illness. [197] Subjects were asked to rate which of a list of life events (e.g., ‘death of a spouse’, ‘divorce’, marital separation’, ‘jail term’, ‘death of a close family member’, ‘personal injury or illness’, ‘marriage’, ‘fired at work’, etc.) had occurred over the past year. The score of "Life Change Units" was summed for each individual. Perceived stress was measured by Perceived Stress Scale which was detailed in 3.2.4.

The Hospital Anxiety and Depression Scale (HADS) [198] was used to test anxiety and depression levels. The HADS is a 14-item scale consisting of 7 items related to anxiety and 7 items related to

depression. Participants rated on a 4-point response scale (from 0 representing absence of symptoms, to 3 representing maximum symptoms), and received a score ranging from 0 to 21 on each of the depression and anxiety subscales. The construction of the HADS avoids the use of somatic symptoms that may confound self-reported measures of depression and anxiety. [199, 200]

Neuroticism was measured by the Neuroticism subscale of the NEO Five-Factor Inventory (NEO-FFI) which is designed to assess personality traits identified in the universal Five-Factor Model (FFM, openness, conscientiousness, extraversion, agreeableness and neuroticism). [201] Participants respond to a series of statements on a 5-point scale ranging from 1 (strongly disagree) to 5 (strongly agree), and a summed score of the 12 items is derived. The neuroticism subscale has high reliability and validity with a Cronbach's α of 0.85. The psychometric properties and cross-cultural applicability of the NEO [201] has been substantiated in a validation study in Singapore. [202]

3.5.4 Enzyme-linked immunosorbent assay (ELISA) and Multiplex analysis

Blood samples were collected during a 4-hour midday period (10:00-14:00 h) which is recommended to test cortisol levels because of the minimal fluctuation during this time. [79] Plasma specimens were prepared for ELISA and Multiplex analysis in 96-well plates. Neuropeptide Y-like immunoreactivity was measured using a competitive enzyme immunoassay (Bachem/Peninsula Laboratories Inc., San Carlos, California). Levels of ACTH and cortisol were determined using the ELISA kits from Alpco Diagnostics (Salem, New Hampshire). Noradrenaline and adrenaline concentrations were determined using commercially available ELISA kits from Abnova (Taipei City, Taiwan). Plates were run according to the manufacturer's instructions.

Plasma concentration of IL-4 was analysed utilizing a custom Milliplex MAP Human Cytokine Magnetic Bead Panel (Millipore Corp., Billerica, Massachusetts) following the kit-specific protocols provided by Millipore. Results were acquired using the Luminex 200 platform (Millipore), with Bioplex Manager 6.0 software, based on standard curves plotted through a 5-parameter logistic curve setting.

3.5.5 Socio-demographic data and clinical profile

Socio-demographic variables included gender, age, family housing type as a proxy for socioeconomic status (1-3 room public housing, 4-5 room public housing or private house), smoking status (non-smoker, past or current smoker), and BMI based on physical measurements of weight and height. Asthma-related clinical assessments consisted of frequency of asthma attack within the past one year, frequency of hospitalization due to asthma within the past one year, duration of history of asthma, and dose of ICS. SRH was assessed as described in 3.2.5.

3.5.6 Statistical analyses

The HPA stress response index was created as a composite index of cortisol, noradrenaline, adrenaline and ACTH using principal component analysis score. Three components were extracted with a percentage of cumulative covariance of 84.467% ($\lambda = 0.9$). The standardized regression factor scores for the three components were summed as HPA stress index based on respective variances explained. Differences in baseline socio-demographic status, asthma-related clinical variables,

asthma control and QoL, psychological variables, HPA index, and the levels of Th2 cytokine IL-4 were compared among groups of participants with acute asthma, chronic asthma, and the healthy controls using 2-tailed Chi-squared test, t-test and One-way ANOVA. Partial correlations (controlling for age) between IL-4 level and psychological and biological stress variables were assessed in the whole sample, asthma and control groups. Dosage of inhaled corticosteroids was further controlled in the correlation between IL-4 and cortisol levels in the whole sample, and acute and chronic asthma groups considering that the cortisol levels in asthma patients may still be affected by past steroid treatment (54/70 patients at baseline in this study). [203] In generalized linear models, the associations of perceived stress, NPY, and HPA index with plasma IL-4 concentration were investigated among participants, controlling for potential confounders (gender, age, ethnicity, smoking status, family housing type, BMI, asthma severity, asthma control, and neuroticism). We evaluated the mediational role of NPY in two sets of regression models predicting plasma levels of IL-4 that related to (1) perceived stress as a predictor variable (psychological stress model) and (2) HPA Index as a predictor variable (biological stress model). We determined the association of perceived stress/HPA Index with IL-4, in the presence and absence of NPY, and evaluated the change in the strength and direction of association, the level of statistical significance and R-squared value. Sobel test was used to evaluate the statistical significance of these mediational tests.

CHAPTER 4 RESULTS

4.1 Study I: Prevalence of anxiety and depressive symptoms in adolescents with asthma: a meta-analysis and meta-regression

Seven hundred and sixty-six abstracts were initially identified through database searches. We excluded 741 articles because they did not meet the inclusion criteria. In the remaining 25 articles, 9 studies failed to include a healthy control group with a sample size of at least 50; 4 studies lacked standardized assessment of depressive and/or anxiety symptoms; 2 studies had the same data with other studies which were already included; 2 studies did not provide sufficient information to calculate an effect size. The selection process of papers was summarized in **Figure 1**. Finally, 8 eligible articles [58, 204-210] were included in this meta-analysis. These studies were mainly conducted in Western countries and involved 3,546 patients and 24,884 healthy controls (**Table 1**).

4.1.1 Aggregate prevalence and pooled odds ratio of depressive symptoms in adolescents with asthma versus the healthy controls

The aggregate prevalence of depressive symptoms among adolescents with and without asthma using the random effects model was 0.27 (95% CI: 0.18-0.39) and 0.13 (95% CI: 0.09-0.19) respectively (**Figure 2A-B**). The risk of developing depressive symptoms was significantly higher in patients with asthma than that in the healthy controls (OR = 2.09, 95% CI: 1.65-2.64, Z = 6.154, $p < 0.001$). **Figure 3A** shows the forest plot generated for the pooled odd ratio of depressive symptoms in the asthma and the control groups from the studies. Statistically significant heterogeneity ($tau^2 = 0.068$, Q = 30.0, df = 7, $p < 0.001$, $I^2 = 76.7$) was present when calculating the OR. Mixed-model meta-regression was conducted to explore the impact of our a priori sources of heterogeneity (demographic

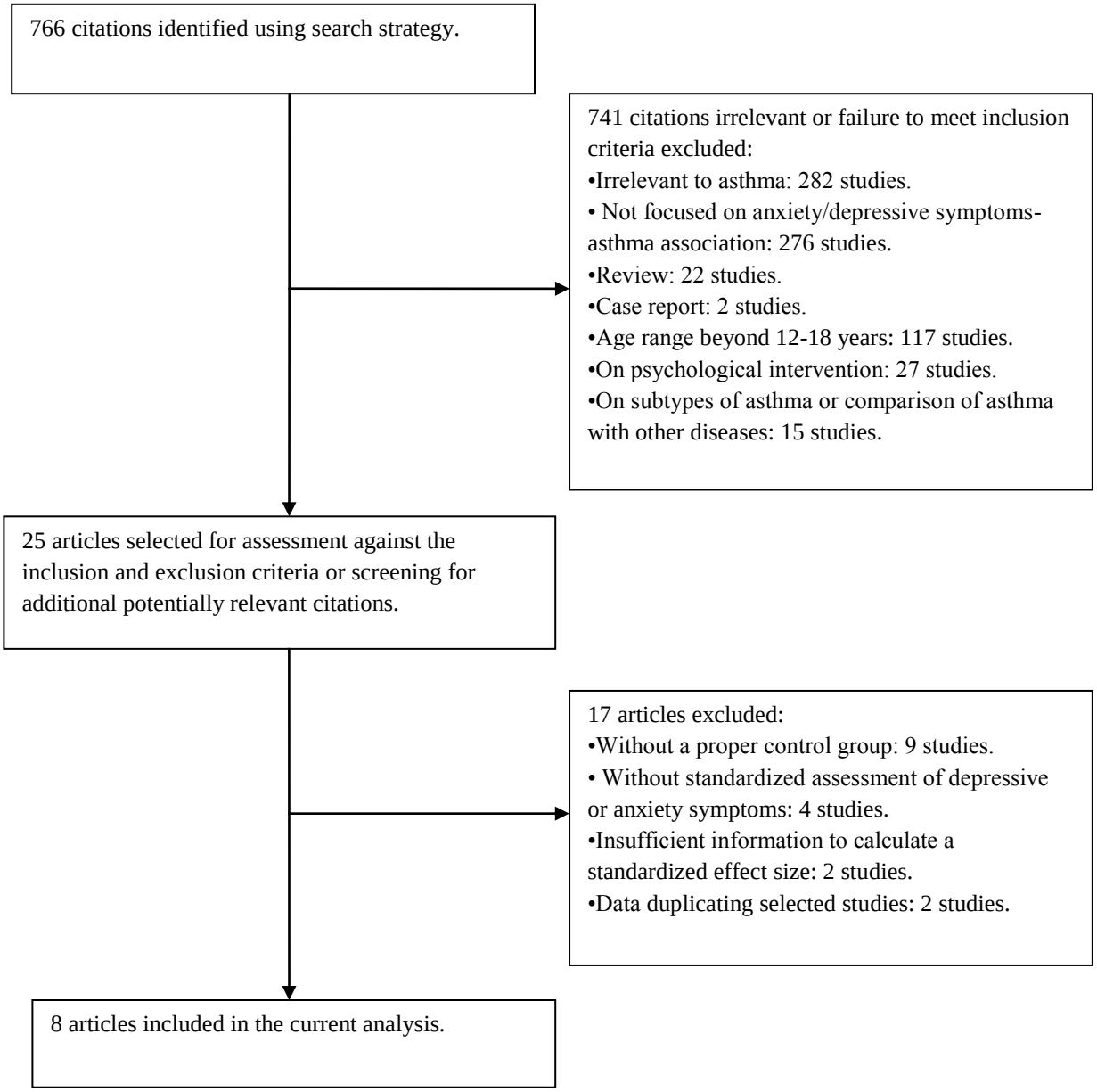


Figure 1. Flowchart describing the process of study selection in meta-analysis

Table 1. Study design and baseline characteristics of studies included in meta-analysis

	Study name and location	Study design	Description of asthma patients	Description of controls	Number of subjects (n, asthma/controls)	Proportio of female in all subjects (%)	Mean age in years (all subjects)	Proportion of Causcasian in all subjects (%)	Depressive/anxiety symptoms assessment method	Prevalence of depressive symptoms (%) asthma/controls	Prevalence of anxiety symptoms (%) asthma/ controls	Proportion of Smokers in all subjects (%)	Proportion of severe asthma in asthma patients (%)
Otten [204]	Across Netherlands	Observational cohort study	Population-based screen for asthma using ISAAC	Population-based sample without asthma	600/4553	52.2	12.97	NA	Depressive Mood List/NA	21.3/10.7	NA	38.5	17.6
Bender [205]	YRBS of US	Observational cohort study	Self-report of asthma diagnosis	All others considered no-asthma	718/13148	51.9	NA	44.0	5 questions from YRBS/NA	45.3/29.3	NA	20.6	NA
Katon [206]	100 clinics in Washington State	Cross-sectional	Parent confirmation of asthma diagnosis and medication	No asthma diagnoses or medication use	781/598	48.3	14.04	6.9	Mood and Feelings Questionnaire/ASI	7.3/4.0	18.7/9.1	4.5	30.3
Goodwin [58]	CHDS in New Zealand	Observational cohort study	Self-report of asthma diagnosis	Those without experience of asthma	386/1650	49.8	NA	NA	CIDI/CIDI	32.4/20.6	21.0/13.6	NA	NA
Gillaspy [207]	A Jobs Corps (Midwestern US)	Case-controlled	25 adolescents with asthma	25 matched controls	25/25	52.0	18.5	36.0	Back Depression Inventory/Back Anxiety Inventory	32.0/8.0	32.0/24.0	NA	NA
Ortega [208]	MECAMDS in Atlanta, New Haven, New York, and Puerto Rico	Cross-sectional	Parental reports of asthma	Youths from selected communities	199/1059	47.0	NA	51.7	DISC/DISC	13.4/11.7	49.2/37.7	NA	NA
Forero [209]	South Western Sydney population study	Case-controlled	Survey of asthma diagnosis	Community sample without asthma	797/3751	51.0	13.52	82.0	HBSC survey instrument/ HBSC survey instrument	42.4/30.4	50.1/35.0	NA	NA
Seigel [210]	New York	Case-controlled	Outpatients	Matched for socioeconomic status	40/100	NA	NA	NA	BDI/NA	62.5/13.0	NA	NA	100.0

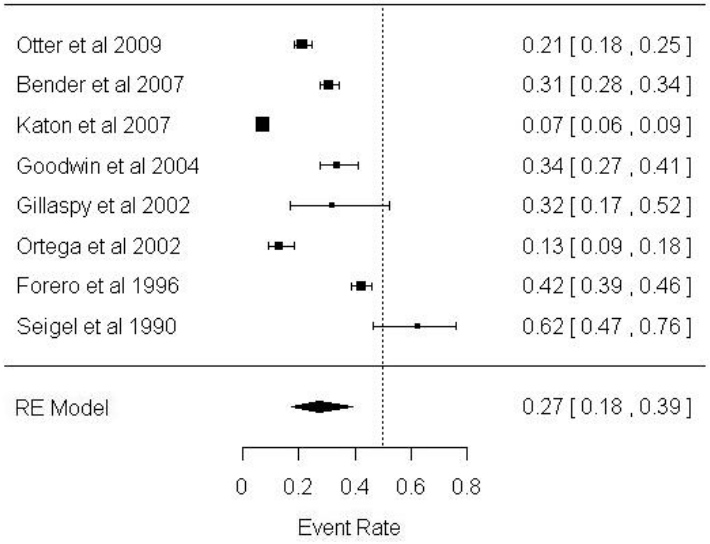
YRBS = Youth Risk Behaviour Survey; CHDS = Christchurch Health and Development Study; MECAMDS = Methods for the Epidemiology of Child and Adolescent Mental Disorders study; ISAAC = Standardized methodology of the International Study of Asthma and Allergy in Childhood; YRBS= Youth Risk Behaviour Survey; ASI = Childhood Anxiety Sensitivity Index; CIDI = Composite International Diagnostic Interview; DISC = Diagnostic Interview Schedule for Children; HBSC = Health Behaviour Among School Children; BDI = Beck Depression Inventory.

and disease-related factors including age, gender, ethnicity, smoking status and severity of asthma) in all studies. Results showed that the mean age (B= -1.444, z = -0.826, p = 0.409) and the proportion of female (B = -3.850, z = -1.815, p = 0.069) of all subjects and the severity of asthma (B = -0.030, z = -0.053, p = 0.958) of asthma patients were non-significant moderators. However, proportions of Caucasian (B = 0.746, z = 2.665, p = 0.008) and smokers (B = 0.875, z = 4.383, p < 0.001) in the sample were significant moderators for heterogeneity (**Table 2**).

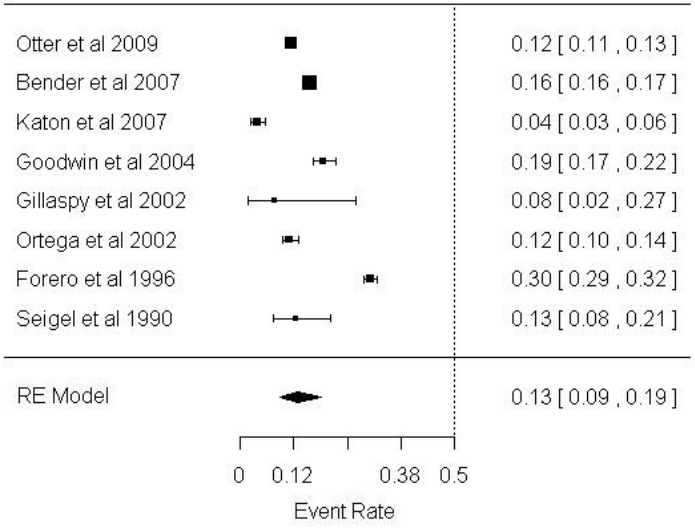
We also tested for the presence of publication biases using funnel plots and Egger's regression test. [211] No significant publication bias was detected (intercept = 1.29, SE = 1.38, 95% CI: -2.08-4.66, t = 0.94, df = 6, p = 0.384) of all studies.

4.1.2 Aggregate prevalence and pooled odds ratio of anxiety symptoms in adolescents with asthma versus the healthy controls

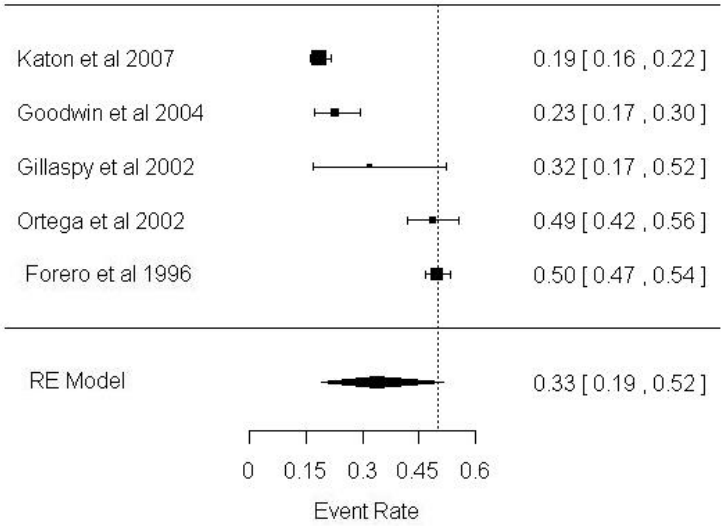
The aggregate prevalence of anxiety symptoms among the asthma patients and the healthy controls was 0.33 (95% CI: 0.19-0.52) and 0.21 (95% CI: 0.12-0.33), respectively (**Figure 2C-D**). Three studies were not used in synthesizing the effect size of the aggregate prevalence of anxiety symptoms as they did not report data on anxiety symptoms. [204, 205, 210] (**Table 1**) The risk of developing anxiety symptoms was significantly higher in asthma patients compared with the healthy controls (OR = 1.83, 95% CI: 1.63-2.07, Z = 9.876, p < 0.001; **Figure 3B**). No significant heterogeneity (tau^2 = 0.000, Q = 3.3, df = 7, p = 0.517, I^2 = 0.0) and no publication bias (intercept = -11.25, SE = 10.26, 95% CI: -43.89-21.39, t = 1.10, df = 3, p = 0.353) was detected in the studies.



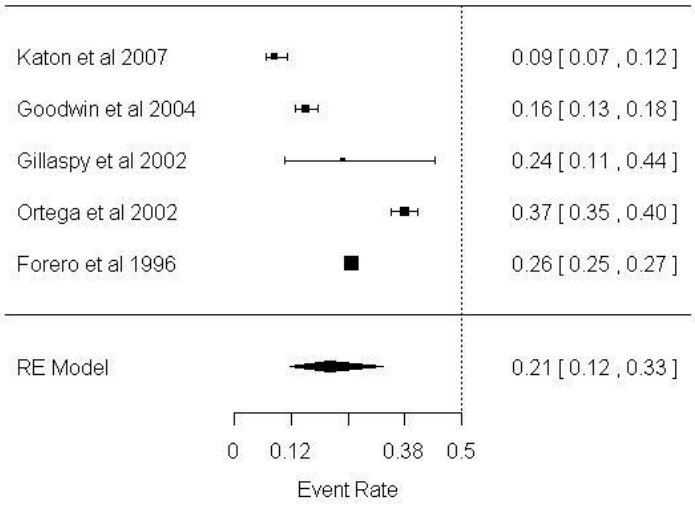
A. Forest plot of the aggregate prevalence of depressive symptoms in adolescents with asthma



B. Forest plot of the aggregate prevalence of depressive symptoms in healthy adolescents

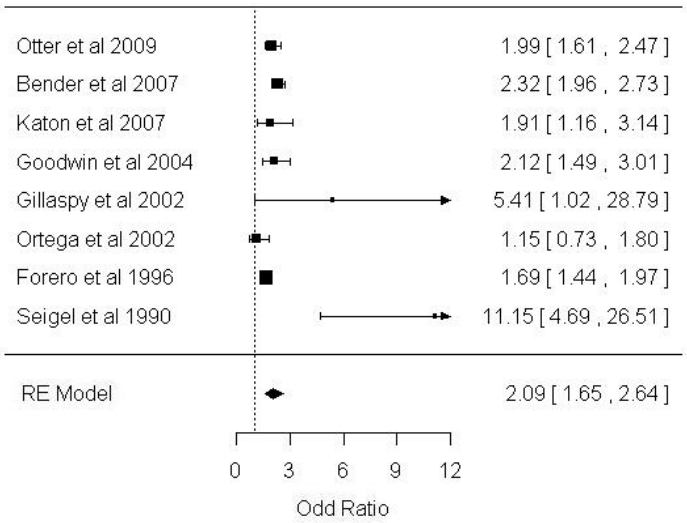


C. Forest plot of the aggregate prevalence of anxiety symptoms in adolescents with asthma

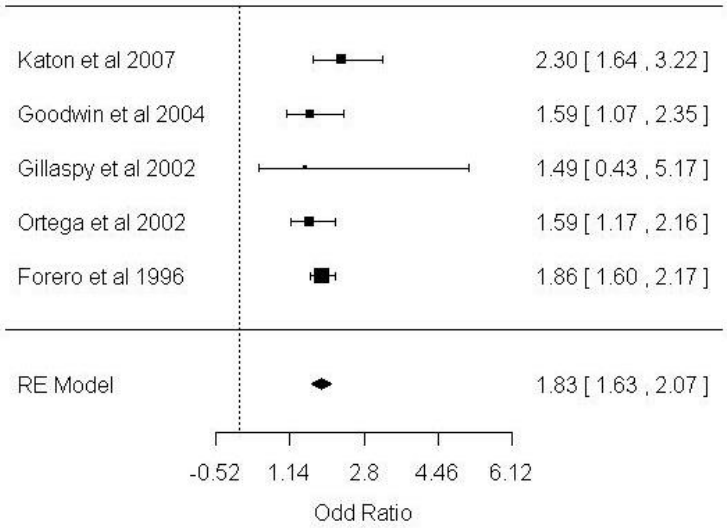


D. Forest plot of the aggregate prevalence of anxiety symptoms in healthy adolescents

Figure 2. Forest plots of the aggregate prevalence of depressive and anxiety symptoms in adolescents with asthma and the healthy controls



A. Forest plot of the pooled odd ratio of depressive symptoms in adolescents with and without asthma



B. Forest plot of the pooled odd ratio of anxiety symptoms in adolescents with and without asthma

Figure 3. Forest plot of the pooled odd ratio of depressive and anxiety symptoms in adolescents with asthma versus the healthy controls

Table 2. Meta-regression analysis of potential moderators to explain heterogeneity of prevalence of depressive symptoms

Factor	Regression Coefficient (SE)	Z Score	Tau^2	df	*p*
Mean Age, years	-1.444 (1.748)	-0.826	0.000	2	0.409
Female, %	-3.850 (2.121)	-0.053	0.005	5	0.958
Caucasian, %	0.746 (0.280)	2.665	0.026	3	0.008
Severe asthma, %	-0.030 (0.563)	-0.053	0.124	1	0.958
Smokers, %	0.875 (0.200)	4.383	0.000	1	<0.001

4.2 Study II: Asthma control, perceived stress, and quality of life in adolescents with asthma: a case-control study

4.2.1 Demographics

There were significant differences in ethnicity ($p < 0.01$) and housing types ($p < 0.01$) among the three groups. Asthma patients had significantly higher proportions of Malays and Indians and were more likely to reside in smaller public housing as compared to the healthy controls. No significant difference was observed in age and gender among the three groups ($p > 0.05$). Higher proportions of smokers ($p < 0.05$), higher BMI ($p < 0.01$) and poorer self-rated health status ($p < 0.01$) were observed among asthma patients, especially those with poorly controlled asthma, when compared to the healthy controls.

4.2.2 Asthma control

Among asthma patients, the mean asthma control score was 22.6 (SD: 1.80) for those with well controlled asthma and 16.2 (SD: 2.78) for those with poorly controlled asthma ($p < 0.001$). Participants with poorly controlled asthma had significantly lower scores ($p < 0.001$) on asthma quality of life (overall and on activity limitation, asthma symptom and emotional function) than those with well controlled asthma. (**Table 3**)

4.2.3 Psychiatric comorbidities

Adolescents with poorly controlled asthma reported significantly more severe panic attacks, depressive symptoms and total internalizing symptoms than respondents with well controlled asthma

and the healthy controls ($p < 0.05$). Respondents with poorly-controlled asthma had significantly more severe generalized anxiety symptoms in comparison to the healthy controls ($p < 0.05$), and more severe obsession and compulsion and total anxiety symptoms than well controlled asthma patients ($p < 0.05$). There was no significant difference in the symptom scores of social phobia and separation anxiety among the three groups ($p > 0.05$). (**Table 3**)

By classifying the number of participants with symptom scores above the borderline and reaching the clinical thresholds of psychiatric comorbidities, the number of subjects who were disturbed by severe panic attacks and depressive symptoms was found to be higher among participants with asthma, especially those with poorly controlled asthma, than their healthy counterparts ($p < 0.05$). No significant difference was observed in social phobia, separation anxiety, generalized anxiety, obsession and compulsion, total anxiety, and total internalizing symptoms among the respondents. (**Table 3**)

To control for confounding variables, we adjusted the mean psychiatric comorbidity score for the difference in gender, age, ethnicity, smoking status and family housing type among the well controlled and poorly controlled asthma and the healthy control groups in analysis of covariance (ANCOVA) models. As shown in **Table 4** (base model), the same difference in scores for panic attacks, depression and total anxiety and internalizing symptoms among the three groups remained statistically significant after adjustment.

4.2.4 Contribution of perceived stress to psychiatric comorbidity

Although no significant difference was observed in the number of stressful life events among the respondents, participants with poorly controlled asthma scored significantly higher on perceived stress than the other two groups ($p < 0.05$). Correlational analyses for the whole sample revealed that perceived stress score was significantly correlated with scores of all psychiatric comorbidities ($p < 0.001$). (**Table 5**) Among asthma patients, asthma control score was negatively correlated with perceived stress and symptom scores of all psychiatric comorbidities ($p < 0.05$), except for social phobia ($p > 0.05$).

Multivariate analyses were performed to determine how much of the difference in symptom scores of panic attacks, depression, total anxiety and total internalizing scales among the well-controlled, poorly controlled asthma and the healthy control groups were explained by the levels of perceived stress. **Table 4** shows the adjusted mean scores for panic attacks, depression, total anxiety and total internalizing symptoms in generalized linear models when perceived stress was added into the model. Perceived stress made a large contribution (R^2 changed from 18% to 29%) to the variations in the severity of psychiatric comorbidities, and its inclusion in the model resulted in substantial decrease in the differences for total anxiety and total internalizing symptoms. No significant differences were found in the scores of total anxiety and total internalizing symptoms between asthma patients and the healthy controls after accounting for the difference in perceived stress.

Among asthma patients, higher asthma control score was associated with lower scores in total anxiety, depression and internalizing symptoms ($p < 0.01$). (**Figure 4**) To evaluate the mediating effect of perceived stress on the relationship between asthma control and severity of psychiatric

comorbidities, we evaluated this relationship after inclusion of perceived stress in the hierarchical model, with covariate adjustment for gender, age, ethnicity, smoking status and family housing type. (**Table 6**) In the presence of perceived stress, asthma control was not significantly associated with psychiatric comorbidities any more except for panic attacks ($p = 0.036$), and perceived stress remained strongly associated with depression, panic attacks, total anxiety and total internalizing symptoms ($p < 0.001$).

Table 3. Socio-demographic characteristics, psychological functioning, clinical profiles and psychiatric comorbidity of adolescents with and without asthma (*n* = 369)

	Poorly controlled (*n* = 61)	Well controlled (*n* = 137)	Healthy control (*n* = 171)	*Significant tests*	*p*
Gender					
Male	36 (59.0)	82 (59.9)	87 (50.9)	$\chi^2 = 2.837$	0.242
Female	25 (41.0)	55 (40.1)	84 (49.1)		
Age (yrs)	15.10 ± 2.75	15.06 ± 2.63	14.90 ± 2.43	$F = 0.208$	0.813
Ethnicity					
Chinese	26 (42.6)	66 (48.2)	113 (66.1)	$\chi^2 = 17.668$	0.001
Malay	19 (31.1)	40 (29.2)	24 (14.0)		
Indian or others	16 (26.2)	31 (22.6)	34 (19.9)		
Smoking status					
Non smoker	52 (85.2)	125 (91.2)	165 (96.5)	$\chi^2 = 9.052$	0.011
Past or current smoker	9 (14.8)	12 (8.8)	6 (3.5)		
Family housing type					
1-2 room public housing	5 (8.2)	2 (1.5)	2 (1.2)	$\chi^2 = 21.168$	0.002
3 room public housing	7 (11.5)	17 (12.6)	30 (17.5)		
4-5 room public housing	45 (73.8)	101 (74.8)	135 (78.9)		
Private housing or detached house	4 (6.6)	15 (11.1)	4 (2.3)		
BMI	21.51 ± 5.55*	21.48 ± 5.79*	19.75 ± 3.95	$F = 5.255$	0.006
Self-rated health	2.96 ± 0.66***+++	3.40 ± 0.61**	3.68 ± 0.68	$F = 28.047$	<0.001
Neuroticism	22.05 ± 7.68*+	18.91 ± 7.50	19.23 ± 7.61	F = 3.722	0.025
No. of stressful life events	2.80 ± 2.49	3.02 ± 2.86	2.57 ± 2.31	F = 1.127	0.325
Perceived stress score	18.25±5.11+	16.15±5.72	17.51±5.39	F = 3.848	0.022
Asthma control score	16.22 ± 2.78	22.64 ± 1.80	NA	t = 15.705	<0.001
Pediatric asthma quality of life	4.51 ± 1.30	6.06 ± 0.85	NA	t = 9.656	<0.001
Activity limitation	4.59 ± 1.42	6.38 ± 0.91	NA	t = 10.348	<0.001
Symptom	4.61 ± 1.40	6.03 ± 0.91	NA	t = 8.224	<0.001
Emotional function	4.34 ± 1.26	5.91 ± 1.01	NA	t = 8.972	<0.001
Social phobia	46.00 ± 12.01	44.65 ± 10.28	44.54 ± 9.95	$F = 0.462$	0.630
No	56 (93.3)	130 (96.3)	163 (95.9)	$\chi^2 = 2.109$	0.701
Borderline	2 (3.3)	4 (3.0)	5 (2.9)		
Yes	2 (3.3)	1 (0.7)	2 (1.2)		
Panic attacks	55.08 ± 18.65***++	48.02 ± 12.44	47.61 ± 10.86	$F = 7.942$	<0.001
No	50 (83.3)	124 (91.2)	155 (90.6)	$\chi^2 = 11.127$	0.025
Borderline	0 (0.0)	3 (2.2)	7 (4.1)		
Yes	10 (16.7)	9 (6.6)	9 (5.3)		
Depression	51.32 ± 15.63**++	45.23 ± 11.85	44.93 ±10.87	$F = 6.694$	0.001
No	49 (81.7)	127 (94.1)	156 (91.8)	$\chi^2 = 9.600$	0.048
Borderline	5 (8.3)	3 (2.2)	9 (5.3)		
Yes	6 (10.0)	5 (3.7)	5 (2.9)		
Separation anxiety	54.54 ± 14.17	50.39 ± 10.47	51.42 ±12.43	$F = 2.460$	0.087
No	47 (79.7)	125 (91.9)	144 (84.2)	$\chi^2 = 6.887$	0.142
Borderline	4 (6.8)	5 (3.7)	11 (6.4)		
Yes	8 (13.6)	6 (4.4)	16 (9.4)		
Generalized anxiety	47.75 ± 13.85*	44.79 ±12.68	42.95 ± 10.12	$F = 3.806$	0.023
No	52 (86.7)	126 (92.6)	165 (96.5)	$\chi^2 = 8.816$	0.066
Borderline	3 (5.0)	6 (4.4)	2 (1.2)		

Yes	5 (8.3)	4 (2.9)	4 (2.3)		
Obsession and compulsion	50.85 ± 11.99+	46.38 ± 11.26	46.19 ± 9.68	F = 4.613	0.011
No	53 (88.3)	128 (94.8)	159 (93.0)	χ^2 = 5.138	0.273
Borderline	3 (5.0)	2 (1.5)	8 (4.7)		
Yes	4 (6.7)	5 (3.7)	4 (2.3)		
Total anxiety symptoms	47.44 ±18.15+	40.07 ± 17.32	42.56 ± 16.37	F = 3.851	0.022
No	51 (86.4)	124 (92.5)	154 (91.1)	χ^2 = 4.575	0.334
Borderline	2 (3.4)	6 (4.5)	7 (4.1)		
Yes	6 (10.2)	4 (3.0)	8 (4.7)		
Total internalizing symptoms	48.46 ± 18.13*++	40.68 ± 15.21	42.47 ± 14.68	F = 5.234	0.006
No	50 (84.7)	125 (93.3)	153 (90.5)	χ^2 = 6.226	0.183
Borderline	2 (3.4)	4 (3.0)	8 (4.7)		
Yes	7 (11.9)	5 (3.7)	8 (4.7)		

Data are presented as n (%) or M±SD. ** $p < 0.01$, * $p < 0.05$ vs. the healthy control group. +++ $p < 0.001$, ++ $p < 0.05$, + $p < 0.05$ vs. the well controlled asthma group. The score of total anxiety symptoms is the sum of the 5 anxiety subscales including panic attacks, social phobia, generalized anxiety, obsession and compulsion, and separation anxiety. Total internalizing symptoms score is the sum of all 6 subscales.

Table 4. Adjusted mean ± standard error symptom scores of psychiatric comorbidity among poorly controlled and well controlled asthma patients, and healthy adolescents: hierarchical regression models

	Poorly controlled (n = 61)	Well controlled (n = 137)	Healthy control (n = 171)	F	p	R^2 change
Base Model: Gender, age, ethnicity, smoking status and family housing type.						
Depression	52.57 ± 1.81**++	47.18 ± 1.53	46.87 ± 1.56	5.152	0.006	
Panic attacks	56.49 ± 1.96***+++	49.77 ± 1.64	50.03 ± 1.68	6.229	0.002	
Social phobia	47.81 ± 1.56	47.21 ± 1.32	47.59 ± 1.34	0.084	0.92	
Separation anxiety	56.92 ± 1.82	53.55 ± 1.52	55.11 ± 1.55	1.649	0.194	
Generalized anxiety	50.05 ± 1.76	47.87 ± 1.48	4.71 ± 1.51	1.722	0.180	
Obsession and compulsions	53.32 ± 1.57	50.02 ± 1.32	50.33 ± 1.35	2.221	0.110	
Total anxiety symptoms	50.76 ± 2.56+	44.33 ± 2.15	48.05 ± 2.19	3.294	0.038	
Total internalizing symptoms	52. 99 ± 2.37++	46.20 ± 2.02	49.01 ± 1.98	4.110	0.017	
Base model plus perceived stress						
Depression	52.56 ± 1.64*	49.22 ± 1.41	47.86 ± 1.38	4.270	0.015	0.256
Panic attacks	56.73 ± 1.86**	52.28 ± 1.60	51.22 ± 1.57	4.632	0.010	0.183
Total anxiety symptoms	48.07 ± 2.26	44.75 ± 1.94	46.48 ± 1.90	1.163	0.314	0.258
Total internalizing symptoms	50.18 ± 2.02	46.34 ± 1.74	47.45 ± 1.70	1.774	0.171	0.287

Figures shown are Bonferroni-adjusted mean ± standard errors estimated from ANCOVA models.
*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. the healthy control group. +++ $p < 0.001$, ++ $p < 0.05$, + $p < 0.05$ vs. the well controlled asthma group.

Table 5. Correlation coefficients of asthma, psychological and psychiatric variables

	Perceived stress score	Asthma control score [+]	Social phobia	Panic attacks	Depression	Separation anxiety	Generalized anxiety	Obsession and compulsion	Total anxiety symptoms	Total internalizing symptoms
Perceived stress Score	1	-0.166	0.501***	0.375***	0.460***	0.395***	0.442***	0.401***	0.528***	0.534***
Asthma control Score		1	-0.087	-0.224	-0.247***	-0.202**	-0.143	-0.165	-0.200	-0.240**
Social phobia			1	0.539***	0.624***	0.538***	0.694***	0.630***	0.822***	0.815***
Panic attacks				1	0.710***	0.504***	0.645***	0.675***	0.650***	0.703***
Depression					1	0.536***	0.660***	0.643***	0.647***	0.774***
Separation anxiety						1	0.566***	0.584***	0.669***	0.693***
Generalized anxiety							1	0.725***	0.789***	0.802***
Obsession and compulsion								1	0.741***	0.756***
Total anxiety symptoms									1	0.954***
Total internalizing symptoms										1

*** $p < 0.0009$ by Holm-Bonferroni correction. ** $p<0.01$

[+] Asthma control score correlations are of asthma patients only.

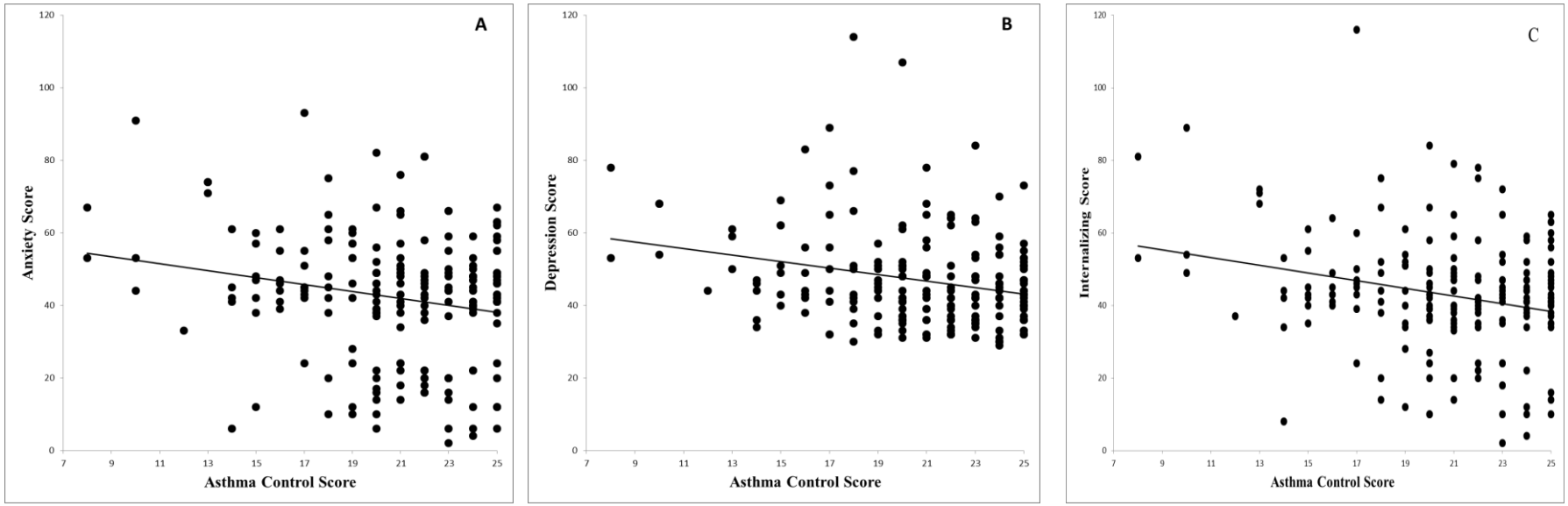


Figure 4. Association of asthma control with psychiatric comorbidity in adolescents with asthma
A) Asthma control and anxiety score; B) Asthma control and depression socre; C) Asthma control and internalizing symptom score.

Table 6. Analyses of perceived stress and asthma control score as predictors of psychiatric comorbidity among asthma patients in regression models (*n* = 198)

	Individual model entry*				Simultaneous model entry*			
	B ± SE	β	*p*	Multiple R^2	B ± SE	β	*p*	Multiple R^2
Dependent variable: Depression								
Independent variables:								
Perceived stress score	1.11 ± 0.15	0.47	<0.001	0.269	1.06 ± 0.16	0.45	<0.001	0.282
Asthma control score	-0.84 ± 0.25	-0.23	0.001	0.114	3.34 ± 1.86	0.16	0.073	
Dependent variable: Panic attacks								
Independent variables:								
Perceived stress score	0.93 ± 0.12	0.39	<0.001	0.208	0.95 ± 0.18	0.36	<0.001	0.193
Asthma control score	-0.87 ± 0.29	-0.22	0.003	0.076	4.62 ± 2.19	0.14	0.036	
Dependent variable: Total anxiety								
Independent variables:								
Perceived stress score	1.63 ± 0.14	0.53	<0.001	0.312	1.67 ± 0.20	0.52	<0.001	0.321
Asthma control score	-0.84 ± 0.35	-0.17	0.018	0.063	3.14 ± 2.43	0.08	0.197	
Dependent variable: Total internalizing								
Independent variables:								
Perceived stress score	1.52 ± 0.13	0.54	<0.001	0.342	1.54 ± 0.19	0. 52	<0.001	0.345
Asthma control score	-0.91 ± 0.32	-0.21	0.005	0.097	3.70 ± 2.21	0.10	0.095	

Data were adjusted for gender, age, ethnicity, smoking status and family housing type.
* Individually: each independent variable (perceived stress score and asthma control score) was entered individually into the regression models; Simultaneously: all independent variables (perceived stress score and asthma control score) were entered simultaneously at the regression model.

4.3 Study III: Asthma, psychological stress and psychiatric comorbidity: a population-based study in adult Singaporeans

4.3.1 Study participants

Among the 2,847 respondents, 144 (5.1%) reported having asthma, of a mean duration of 12.2 (SD: 11.1) years, and mean number of doctor visits of 2.3 (SD, 4.2, range: 0-30) in the last 12 months; 8 of them (5.6%) reported having been hospitalized for 1 to 3 episodes of asthma in the last 12 months. Consistent with our previous findings, [165, 212] participants with asthma tended to be younger, and were more likely to be Malay and less likely to be Chinese. A total of 843 (29.6%) participants reported having other chronic physical conditions (10.3% hypertension, 8.2% lipid abnormalities, 6.4% diabetes, 2.6% coronary and heart conditions, 4.1% arthritis, 1.5% cataract, 0.2% chronic obstructive pulmonary disease, 0.5% stroke, 0.3% hip fracture, 0.6% cancer, 0.2% kidney failure, 0.8% urinary disorders, and 12.8% others). A total of 1860 respondents reported no chronic physical illnesses (65.3%). The demographic profiles of the respondents are shown in **Table 7** by groups of asthma (A), other chronic physical conditions (B), and no chronic physical condition (C).

A total of 520 (18.3%) individuals had a score of GHQ $\geq$ 2 and were further administered SCAN. Case-level diagnosis of any psychiatric disorder was made for 7.3% of respondents (n = 208), MDD for 5.8% (n = 164), and GAD for 3.1% (n = 89). A total of 311 respondents reported 1 to 2 stressful life events (10.9%) and another 82 respondents reported having 3 or more stressful life events (2.9%) in the previous 6 months.

4.3.2 Comorbidity of psychiatric disorders with asthma and other chronic physical conditions

Table 7. Socio-demographic and clinical characteristics of adults aged 20-59 by asthma, other chronic physical conditions, and no chronic physical conditions groups in Singapore (National Mental Health Survey 2003)

	Asthma (A)	Other Chronic Physical Conditions (B)	No Chronic Physical Conditions (C)	Significance Tests	
				(A) vs (B) vs (C)	*p*
No. of respondents	(*n* = 106)	(*n* = 843)	(*n* = 1860)		
Female sex	80 (75.5)	513 (60.9)	1174 (63.1)	χ^2 = 8.73, 2df	0.013
Age					
20-34	45 (42.5)	98 (11.6)	649 (34.9)		
35-44	29 (27.4)	266 (31.6)	748 (40.2)		
45-59	32 (30.2)	479 (56.8)	463 (24.9)	χ^2 = 305.06, 4df	<0.001
Ethnicity					
Malay	47 (44.3)	243 (28.8)	656 (35.3)		
Chinese	19 (17.9)	290 (34.4)	600 (32.3)		
Indian	40 (37.7)	310 (36.8)	604 (32.5)	χ^2 = 22.41, 4df	<0.001
Employment					
Employed	70 (66.0)	496 (58.8)	1173 (63.1)		
Housewives, retired, students	36 (34.0)	347 (41.2)	687 (36.9)	χ^2 = 5.19, 2df	0.075
Marital status					
Married	76 (71.7)	655 (77.7)	1410 (75.8)		
Unmarried	30 (28.3)	188 (22.3)	450 (24.2)	χ^2 = 2.39, 2df	0.303
Formal Education					
No education	5 (4.7)	46 (5.5)	52 (2.8)		
Primary to secondary	77 (72.6)	626 (74.3)	1257 (67.6)		
Post-secondary and tertiary	24 (22.6)	171 (20.3)	551 (29.6)	χ^2 = 35.06, 4df	<0.001
No. of coexisting chronic illness					
0-2	106 (100.0)	746 (88.5)	1860 (100.0)		
3 or more	0 (0.0)	97 (11.5)	0 (0.0)	χ^2 = 234.31, 2df	<0.001

Figures in table denote number and %.

Table 8. Prevalence (%) of psychiatric disorders and stressful life events, by asthma, other chronic physical conditions and no chronic physical conditions groups

	Asthma (A)	Other Chronic Physical Conditions (B)	No Chronic Physical Conditions (C)	Significance Tests	*p*
Any psychiatric disorder	15 (14.2)	105 (12.5)	88 (4.7)	χ^2 = 57.79, 2df	<0.001
Major depression disorder	14 (13.2)	82 (9.7)	68 (3.7)	χ^2 = 49.78, 2df	<0.001
Generalized anxiety disorder	7 (6.6)	40 (4.7)	42 (2.3)	χ^2 = 15.93, 2df	<0.001
No of life events					
1-2	17 (16.0)	134 (15.9)	160 (8.6)		
3 or more	15 (14.2)	30 (3.6)	37 (2.0)	χ^2 = 91.48, 4df	<0.001
Type of life events					
Threat	7 (6.6)	58 (6.9)	53 (2.8)		
Loss	5 (4.7)	32 (3.8)	51 (2.7)		
Mixed	19 (17.9)	74 (8.8)	93 (5.0)	χ^2 = 68.94, 4df	<0.001

Figures in table denote number and %.

Table 9. Association of asthma and other chronic physical conditions with measures of coexisting psychiatric disorders and stressful life events

	Controlling for life events	Asthma vs Other Chronic Physical Conditions			Asthma vs No Chronic Physical Conditions			Other Chronic Physical Conditions vs No Chronic Physical Conditions		
		OR	(95% CI)	*p*	OR	(95% CI)	*p*	OR	(95% CI)	*p*
Number of life events (vs. none)										
1-2	NA	1.16	0.66-2.06	0.605	2.32	1.33-4.06	0.003	1.99	1.52-2.62	<0.001
3 or more	NA	4.33	2.09-8.95	<0.001	7.64	3.87-15.06	<0.001	1.77	1.01-3.11	0.049
Any psychiatric disorder	No	1.17	0.63-2.18	0.622	2.86	1.54-5.28	0.001	2.44	1.75-3.42	<0.001
	Yes	0.66	0.32-1.38	0.269	1.27	0.61-2.63	0.528	1.91	1.32-2.76	0.001
Major depressive disorder	No	1.46	0.76-2.79	0.255	3.54	1.86-6.72	<0.001	2.43	1.66-3.55	<0.001
	Yes	0.95	0.46-1.98	0.886	1.77	0.85-3.70	0.129	1.87	1.25-2.79	0.002
Generalized anxiety disorder	No	1.58	0.66-3.79	0.303	2.51	1.06-5.92	0.036	1.58	0.95-2.63	0.076
	Yes	1.09	0.43-2.76	0.861	1.30	0.51-3.33	0.588	1.19	0.71-2.01	0.504

* Odds ratios were adjusted for sex, age, ethnicity, employment status, marital status, education, number of coexisting chronic illnesses.

As shown in **Table 8**, there were significantly greater prevalence of any psychiatric disorder, MDD and GAD among respondents with asthma (A) and other chronic physical conditions (B), compared to respondents with no chronic physical conditions (C). The odds ratio of association with any psychiatric disorder, MDD and GAD for A versus C, and with any psychiatric disorder and MDD for B versus C remained significantly elevated (OR point estimates ranging from 2.4 to 3.5) after adjusting for group differences in sex, age, ethnicity, employment status, marital status, education, and number of coexisting chronic medical conditions. (**Table 9**) Only the OR of association with GAD for B versus C was not significant after adjusting for potential confounders (OR = 1.58, 95% CI: 0.95-2.63). (**Table 9**)

4.3.3 Stressful life events as a mediating factor for psychiatric comorbidity

Considerably greater numbers of stressful life events were reported among respondents with asthma (14.2% with 3 or more stressful life events), much more than those reported by respondents with other chronic physical conditions (3.6%) and without chronic physical conditions (2.0%). Participants with asthma were found to have a high proportion of mixed threat and loss type of life events. (**Table 8**) Notably, respondents with asthma were 4 times more likely to report 3 or more life events compared to respondents with other chronic physical conditions (adjusted OR = 4.33, 95% CI: 2.09-8.95, $p < 0.001$) (**Table 9**).

We determined in hierarchical models whether stressful life events explained to a greater extent the comorbidity of psychiatric disorders in asthma than it did for other chronic physical conditions (**Table 9**). The addition of the frequency of stressful life events into the models substantially reduced

the significance of the OR estimates for asthma versus no chronic physical conditions for comorbidity of MDD (OR decreased from OR = 3.54, 95% CI: 1.86-6.72, $p < 0.001$ to OR = 1.77, 95% CI: 0.85-3.70, $p = 0.129$), GAD (OR decreased from OR = 2.51, 95% CI: 1.06-5.92, $p = 0.036$ to OR = 1.30, 95% CI: 0.51-3.33, $p = 0.588$) and any psychiatric disorder (OR decreased from OR = 2.86, 95% CI: 1.54-5.28, $p < 0.001$ to OR = 1.27, 95% CI: 0.61-2.63, $p = 0.528$). The OR estimates for other chronic physical conditions (B) versus no chronic physical conditions (C) were found to remain significantly elevated. Consistently, Sobel test (**Table 10**) revealed that the presence of stressful life events, particularly 3 or more stressful life events, mediated the relationship between asthma and co-occurring case diagnosis of any psychiatric disorder, MDD and GAD. On the other hand, the mediational effect of stressful life events was not found for the association between other chronic physical conditions and psychiatric disorders ($p > 0.05$).

4.3.4 Relative contribution of stressful life events and concurrent psychiatric disorders to impaired quality of life

Next, we compared QoL scores among the study groups, and examined the relative contributions of stressful life events, any psychiatric disorder, MDD and GAD in hierarchical models to the differences in this relationship (**Table 11**). In the base model controlling for potential confounders, QoL scores were significantly lower for respondents with asthma (A) and with other chronic physical conditions (B), compared with those without chronic physical conditions (C) ($p < 0.001$). The difference in QoL between asthma (A) and no chronic physical conditions (C) virtually disappeared after accounting for number of stressful life events, but not after accounting for other psychiatric variables. The difference in QoL between groups with other chronic physical conditions (B) and without chronic physical conditions (C) remained significant even after adjusting for number of

stressful life events and other psychiatric variables.

Table 10. Sobel test of the mediation of stressful life events on the coexistence of psychiatric disorders with asthma andwith other chronic physical conditions

	Asthma vs Other Chronic Physical Conditions		Asthma vs No Chronic Physical Conditions		Other Chronic Physical Conditions vs No Chronic Physical Conditions	
	z	*p*	z	*p*	z	*p*
Any psychiatric disorder						
1-2 life events	0.030	0.976	2.507	0.012	1.701	0.089
3 or more life events	4.210	<0.001	6.317	<0.001	0.937	0.349
Major depressive disorder						
1-2 life events	0.030	0.976	2.508	0.012	1.567	0.117
3 or more life events	4.131	<0.001	6.010	<0.001	0.909	0.363
Generalized anxiety disorder						
1-2 life events	0.026	0.979	2.466	0.014	1.435	0.151
3 or more life events	2.291	0.022	5.361	<0.001	0.732	0.464

Sobel test of the indirect effect is given by a ratio which is considered as a Z test. $p < 0.05$ is considered significant mediating effect.

Table 11. Quality of life among asthma, other chronic physical conditions and no chronic physical conditions groups, and relative contributions from stressful life events and coexisting psychiatric disorders

Hierarchical models	R^2 Change	SF-12 Mental Component Summary score, adjusted mean and SE					Estimated Mean Difference ± SE	
		Asthma (A)	Other Chronic Physical Conditions (B)	No Chronic Physical Conditions (C)	*F* (2,2844),	*p*	A minus B	A minus C
Base Model	NA	50.34 ± 0.75***	51.60 ± 0.43***	52.81 ± 0.44	12.93	<0.001	-1.25 ± 0.70	-2.47 ± 0.68
Plus No. of life events	0.162	47.39 ± 0.70	47.30 ± 0.44*	47.98 ± 0.45	3.12	0.044	0.08 ± 0.65	-0.59 ± 0.62
Plus Any psychiatric disorder	0.105	47.85 ± 0.72*	48.95 ± 0.43*	49.65 ± 0.45	6.10	0.002	-1.10 ± 0.66	-1.80 ± 0.64
Plus Major depressive disorder	0.077	47.80 ± 0.74*	48.76 ± 0.45*	49.59 ± 0.47	6.83	0.001	-0.95 ± 0.67	-1.78 ± 0.65
Plus Generalized anxiety disorder	0.029	47.60 ± 0.79**	48.72 ± 0.52***	49.84 ± 0.54	11.24	<0.001	-1.11 ± 0.69	-2.24 ± 0.67

Base model: adjusted for sex, age, ethnicity, employment status, marital status, education, number of coexisting chronic illness.
*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ versus no chronic physical conditions group.

4.4 Study IV: The impact of stressful life events on quality of life in the elderly with airway obstruction

This study consisted of 497 participants with the average age of 72.1. Among the respondents, 32.9% had airway obstruction, and 67.1% had no airway obstruction. The proportion of respondents with stressful life events in the past one year was 43.4%. The socio-demographic and psychological characteristics of study participants with and without airway obstruction are shown in **Table 12**. Participants with airway obstruction had higher mean age ($t = 3.328$, $p = 0.001$), higher proportion of smokers ($\chi^2 = 20.586$, $df = 3$, $p < 0.001$) and increased number of diseases ($t = 7.096$, $p < 0.001$). No significant difference in the number of stressful life events or perceived stress was observed between airway obstructive and non- airway obstructive participants ($p > 0.05$).

Table 12 shows that compared with those without airway obstruction, respondents with airway obstruction had decreased post-bronchodilator FEV1 ($t = 6.185$, $p < 0.001$), MMSE score ($t = 3.090$, $p = 0.002$) and SF-36 PCS ($t = 2.726$, $p = 0.007$) score. There was no difference in CFQ or GDS depression scores between participants with and without airway obstruction ($p > 0.05$) in the whole sample. Next, we evaluated the effect of stressful life events on GDS, CFQ, MMSE, SF-36 PCS and MCS scores by subgroups of participants with and without airway obstruction. As shown in **Table 13**, two-way ANCOVA showed significant main effects of stressful life events for GDS ($F = 64.500$, $df = 1$, $p < 0.001$), SF-36 PCS ($F = 7.054$, $df = 1$, $p = 0.008$) and MCS ($F = 14.710$, $df = 1$, $p < 0.001$) scores. Significant interactions of stressful life events with airway obstruction were found for variables of GDS ($F = 10.970$, $df = 1$, $p = 0.001$), SF-36 PCS ($F = 4.055$, $df = 1$, $p = 0.045$), and MCS ($F = 4.538$, $df = 1$, $p = 0.034$) scores. The simple effects of stressful life events on GDS, SF-36 PCS and MCS are shown for participants with and without airway obstruction respectively in **Figure**

5. Higher stress score was associated with higher GDS score, lower SF-36 PCS and MCS scores in participants with airway obstruction, more than in those without airway obstruction after adjusting for potential confounders, indicating the association of stressful life events with more psychological symptoms and poor QoL among individuals with airway obstruction, in comparison to non- airway obstructive individuals. The other significant main effect observed was of airway obstruction for decreased post-bronchodilator FEV1 ($F = 17.458$, $df = 1$, $p < 0.001$) and higher CFQ scores ($F = 5.424$, $df = 1$, $p < 0.05$) after adjustment of sex, age, ethnicity, smoking status, and number of chronic illness, indicating impaired pulmonary and cognitive functions in respondents with airway obstruction.

Table 12. Socio-demographic, pulmonary and psychological variables of study participants aged 65 or older (Singapore Longitudinal Aging Study, SLAS-2)

	Airway Obstruction		Non- Airway Obstruction		Significant Test	*p*
	n	%	n	%		
Total	136	32.9	277	67.1		
Sex						
Male	58	42.6	112	40.7	$\chi^2 = 0.138$, df = 1	0.710
Female	78	57.4	163	59.3		
Age (years, M±SD)	73.19	±5.87	71.23	±5.47	t = 3.328	0.001
Ethnicity						
Chinese	121	89.0	241	87.6	$\chi^2 = 0.154$, df = 1	0.694
Non-Chinese	15	11.0	34	12.4		
Smoking						
Never smoker	77	57.6	211	77.3	$\chi^2 = 20.59$, df = 3	<0.001
Past smoker	29	21.6	41	15.0		
Current smoker < 10 cigarettes daily	18	13.4	12	4.4		
Current smoker ≥ 10 cigarettes daily	10	7.5	9	3.3		
Number of chronic diseases	2.60	±1.30	1.65	1.25	t = 7.096	<0.001
Number of Stressful Life Events	0.67	±0.98	0.74	±1.11	t = 0.609	0.543
Stressful Life Event Score	1.02	±1.71	1.12	±1.78	t = 0.525	0.600
Post-bronchodilator FEV1	1.42	±0.50	1.75	±0.52	t = 6.185	<0.001
CFQ Score	38.0	±9.21	38.8	±9.83	t = 0.733	0.464
MMSE Score	26.4	±3.26	27.4	±3.11	t = 3.090	0.002
GDS Depression score	1.13	±2.04	0.84	±1.69	t = 1.487	0.138
GDS ≥ 5, % (n)	5.2	(7)	2.9	(8)	$\chi^2 = 1.349$, df = 1	0.245
SF-36 PCS	44.6	±9.04	47.1	±7.21	t = 2.726	0.007
SF-36 MCS	54.9	±7.83	55.1	±6.68	t = 0.353	0.724

Figures in table denote mean ± SD or number and %. FEV1 = forced expiratory volume in the first second; CFQ = Cognitive Failure Questionnaire; MMSE = Mini-Mental State Examination; GDS = the Geriatric Depression Scale; SF-36 = 36-Item Short-Form Healthy Survey; PCS = Physical Health Component Summary; MCS = Mental Health Component Summary.

Table 13. Two-way ANCOVA: stressful life events, airway obstruction and mental and physical variables

	Main Effects of Airway Obstruction (df = 1)		Main Effects of Stress (df = 1)		Interaction (df = 1)	
	F	*p*	F	*p*	F	*p*
Post-bronchodilator FEV1	17.458	<0.001	0.323	0.570	2.057	0.152
CFQ Score	5.424	0.020	1.927	0.166	2.514	0.114
MMSE Score	1.799	0.181	1.159	0.282	0.380	0.538
GDS Depression score	2.353	0.126	64.500	<0.001	10.970	0.001
SF-36 PCS	0.432	0.512	7.054	0.008	4.055	0.045
SF-36 MCS	0.659	0.417	14.710	<0.001	4.538	0.034

Adjusted variables: sex, age, ethnicity, smoking status, and number of chronic illness. FEV1 = forced expiratory volume in the first second; CFQ = Cognitive Failure Questionnaire; MMSE = Mini-Mental State Examination; GDS = the Geriatric Depression Scale; SF-36 = 36-Item Short-Form Healthy Survey; PCS = Physical Health Component Summary; MCS = Mental Health Component Summary.

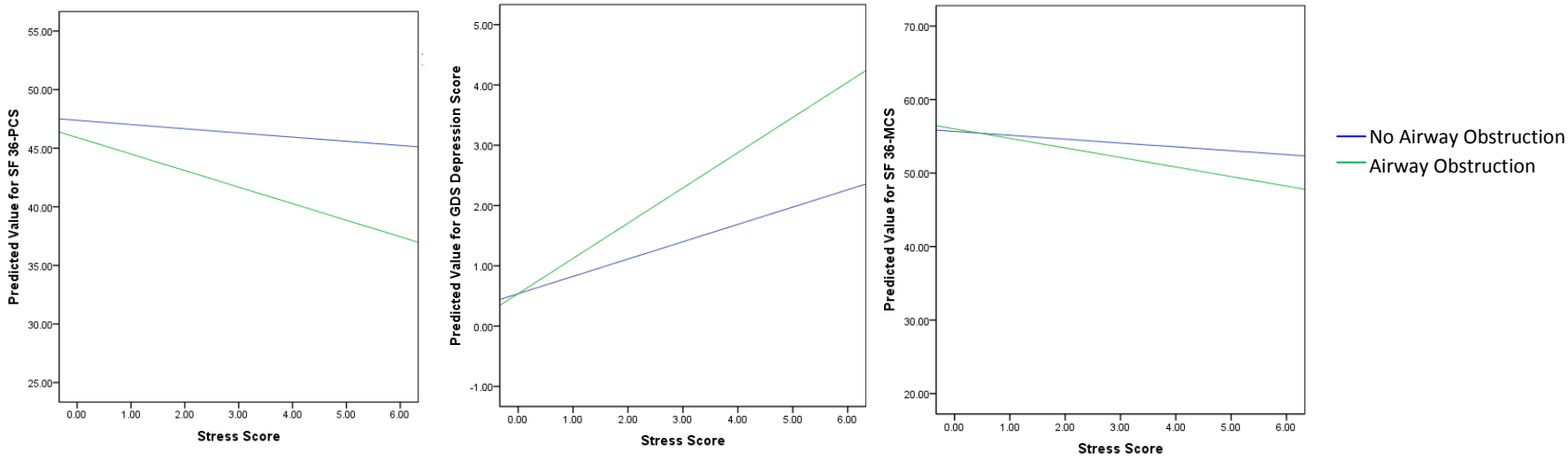


Figure 5. Stressful life events and mental and functional well-being among study participants with or without airway obstruction

SF-36 = 36-Item Short-Form Healthy Survey; PCS = Physical Health Component Summary; GDS = the Geriatric Depression Scale; MCS = Mental Health Component Summary.

4.5 Study V: Stress, neuropeptide Y, and young adult asthma: a follow-up study

Table 14 shows the baseline demographic, clinical, and psychological profiles of study participants aged 21-35 by acute asthma, stable asthma and the healthy control groups. There were no significant differences in mean age, proportions of gender and family housing conditions among the three groups ($p > 0.05$). Participants with acute asthma were more likely smokers ($p < 0.001$), to report poorer self-rated health status ($p < 0.001$), and have lower levels of lung function (FEV1 Pred and FVC Pred, $p < 0.01$). Compared to patients with stable asthma, patients with acute asthma had significantly longer mean duration of asthma history of 18.9 versus 15.1 years ($p < 0.001$); they did not have significantly more numbers of asthma attacks, but they were more frequently hospitalized in the last 12 months. Six (46.2%) patients in the acute asthma group and 20 (42.6%) patients in the stable asthma group reported taking medium to high dose of ICS ($p = 0.82$). Patients with acute asthma gave lower asthma control score (13.23 ± 5.12 vs. 18.86 ± 4.75, $p < 0.001$), and poorer asthma-related quality of life (3.94 ± 1.49 vs. 5.10 ± 1.35, $p < 0.01$). Asthma patients, especially those with acute asthma, reported higher levels of anxiety, depression, and neuroticism (a personality trait closely related to psychological distress). [57]

No significant difference in stressful life event score was observed among the three participant groups (164.14 ± 133.31 vs. 144.57 ± 101.72 vs. 139.39 ± 89.03, $p > 0.05$). However, notably higher scores of perceived stress were reported among respondents with acute asthma, than those reported by the healthy controls (21.43 ± 6.02 vs. 17.53 ± 6.20 vs. 16.58 ± 6.71, $p < 0.05$). They also had higher levels of ACTH (6.67 pmol/L vs. 4.12 pmol/L vs. 3.60 pmol/L, $p < 0.05$), noradrenaline (645.04 pg/ml vs. 498.8 pg/ml vs. 346.1 pg/ml, $p < 0.001$), adrenaline (99.6 pg/ml vs. 52.3 pg/ml vs. 31.7 pg/ml, $p < 0.001$), and the HPA index (0.34 vs. -0.00 vs. -0.09 , $p < 0.01$) and lower levels of

cortisol (387.1 nmol/L vs. 389.4 nmol/L vs. 417.1 nmol/L, $p < 0.01$) than the healthy controls, indicating higher levels of stress and dysregulated HPA axis responsiveness in asthma patients.

Respondents with asthma, especially acute asthma, had higher levels of Th2 cytokine IL-4 than healthy controls (6.96 pg/ml vs. 3.63 pg/ml vs. 1.57 ng/ml, $p < 0.001$), and lower levels of NPY (0.42 ng/ml vs. 0.47 ng/ml vs. 0.62 ng/ml, $p = 0.01$). (**Table 15**)

In the whole sample, baseline IL-4 level, controlling for age, was significantly correlated with NPY ($p < 0.05$), ACTH ($p < 0.01$), noradrenaline ($p < 0.05$), adrenaline ($p < 0.001$), HPY index ($p < 0.01$), perceived stress ($p < 0.05$), anxiety ($p < 0.05$) and neuroticism ($p < 0.05$). Among healthy controls, IL-4 level was significantly correlated with noradrenaline ($p = 0.01$) and HPA index ($p < 0.05$). Among patients with acute asthma, but not chronic asthma, IL-4 was significantly correlated with adrenaline ($p < 0.05$), HPA index ($p = 0.001$), perceived stress ($p < 0.05$), and neuroticism ($p = 0.01$). IL-4 was not significantly correlated with NPY among patients with acute asthma, but was significantly correlated ($p < 0.001$) among patients with chronic asthma. **Table 16**.

Among asthma patients, perceived stress ($p < 0.05$) and plasma NPY level ($p < 0.001$) in separate models (1 and 2) were individually significant predictors of IL-4 levels at baseline, controlling for gender, age, ethnicity, smoking status, family housing type, BMI, asthma severity, asthma control, and neuroticism. In the combination model that included both perceived stress and NPY (Model 3), only NPY remained a significant predictor of IL-4, whereas there was a reduction in the strength and significance of the association between perceived stress and IL-4. The Sobel test indicated that NPY

was a significant mediating variable in the relationship between perceived stress and baseline level ($Z = 2.134$, $p < 0.05$) of IL-4. In the biological stress model that assessed the HPA Index measure of biological stress, HPA index and NPY were also individually significant predictors of IL-4 levels in separate models, but in the combination model, both HPA index and NPY remained independently associated with IL4 levels. There was no suggestion from the Sobel test that NPY significantly mediated the relationship between HPA index and IL-4.

Further analyses showed that perceived stress measured at baseline significantly predicted IL-4 level one year later, and this was not independent of NPY. The Sobel test indicated that NPY was a significant mediating variable ($Z = 2.134$, $p < 0.05$). HPA index at baseline, however, failed to predict IL-4 level one year later. In both analyses, NPY at baseline remained a significant independent predictor of IL-4 at one year. **Table 17**.

Table 14. Socio-demographic, clinical, and psychological characteristics of young adults aged 21-35 years by acute asthma, chronic asthma, and healthy control

	Acute Asthma (n = 19)	Chronic Asthma (n = 51)	Healthy Control (n = 69)	Significance Tests	
				F/ χ^2/ t	p
Gender					
Male	10 (52.6)	26 (51.0)	37 (53.6)	0.082	0.960
Female	9 (47.4)	25 (49.0)	32 (46.4)		
Age	27.00 ± 4.89	27.27 ± 5.14	25.48 ± 3.38	2.800	0.064
Housing type					
1-3 room public housing	6 (40.0)	14 (27.5)	23 (33.3)	0.984	0.612
4-5 room public housing and others	9 (60.0)	37 (72.5)	46 (66.7)		
Smoking status					
Non-smoker	6 (40.0)	35 (68.6)	65 (94.2)	26.216	<0.001
Past or current smoker	9 (60.0)	16 (31.4)	4 (5.8)		
Self-rated health					
Very poor to fair to good	15 (93.8)	44 (86.3)	27 (39.1)	35.298	<0.001
Very good to excellent	1 (6.3)	7 (13.7)	42 (60.9)		
FEV1	2.04 ± 1.11*	2.79 ± 0.78	2.82 ± 1.03	3.043	0.051
FEV1 Pred (%)	58.30 ± 25.64**+	85.34 ± 21.73	87.21 ± 28.88	5.377	0.006
FVC	2.76 ± 1.37	3.56 ± 1.02	3.70 ± 1.41	2.375	0.097
FVC Pred (%)	67.20 ± 26.25**+	90.34 ± 20.45	95.81 ±31.03	4.886	0.009
Number of asthma attack within 1 year	4.44 ± 2.34	3.84 ± 5.87	NA	0.155	0.695
Number of hospitalization past year)	1.81 ± 1.56	0.53 ± 0.95	NA	16.062	<0.001
Duration of asthma (years)	18.87 ± 10.48	15.08 ± 10.19	NA	86.237	<0.001
Dose of Inhaled corticosteroids (ICS)					
Medium to high dose (≥250 μg/day)	6 (46.2)	20 (42.6)	NA	0.390	0.823
Low dose (<250 μg/day)	2 (15.4)	11 (23.4)	NA		
No use	5 (38.5)	16 (34.0)	NA		
Asthma control	13.23 ± 5.12	18.86 ± 4.75	NA	14.061	<0.001
Asthma quality of life	3.94 ± 1.49	5.10 ± 1.35	NA	7.803	0.007
Activity limitation	1.97 ± 0.83	2.42 ± 0.64	NA	4.544	0.037
Symptom	3.74 ± 1.41	5.50 ± 1.48	NA	9.198	0.004
Emotional function	3.91 ± 1.72	5.01 ± 1.61	NA	5.258	0.025
Exposure	3.87 ± 1.51	5.05 ± 1.54	NA	6.873	0.011
Stressful life events	164.14 ± 133.31	144.57 ± 101.72	139.39 ± 89.03	0.364	0.695
Perceived stress	21.43 ± 6.02*	17.53 ± 6.20	16.58 ± 6.71	3.292	0.040
Neuroticism	26.13 ± 8.16**	21.31 ± 6.99	19.16 ± 8.06	5.270	0.006
Anxiety	9.13 ± 3.27*	7.47 ± 3.77	6.41 ± 3.54	3.944	0.022
Depression	5.67 ± 3.48**	4.06 ± 2.81	3.26 ± 2.49	5.065	0.008

Figures in table denote M ± SD or number and %. ** $p < 0.01$, * $p < 0.05$ vs. the healthy control group. + $p < 0.05$ vs. the chronic asthma group. FEV1 Pred = forced expiratory volume in 1 second predicted, FVC Pred = forced vital capacity predicted.

Table 15. Measurements of IL-4 and stress-related variables in young adults aged 21-35 years by acute asthma, chronic asthma, and healthy control

	Acute Asthma (n = 19)	Chronic Asthma (n = 51)	Healthy Control (n = 69)	Significance Tests	
				F	p
IL-4 (pg/ml)	6.96 ± 7.15***	3.63 ± 6.79	1.57 ± 2.27	8.914	<0.001
NPY (ng/ml)	0.42 ± 0.14*	0.47 ± 0.24*	0.62 ± 0.40	4.786	0.010
Cortisol (nmol/L)	387.11 ± 130.63	389.44 ± 246.32*	517.10 ± 256.58	4.876	0.009
ACTH (pmol/L)	6.67 ± 10.96*	4.12 ±3.55	3.60 ± 1.75	3.172	0.045
Noradrenaline (pg/ml)	645.04 ± 329.46**	498.77 ± 413.41*	346.15 ± 179.90	8.615	<0.001
Adrenaline (pg/ml)	99.64 ± 76.79***+++	52.28 ± 43.92*	31.74 ± 18.51	20.889	<0.001
HPA Index	0.34 ± 0.51*	-0.00 ±0.59	-0.09 ± 0.37	5.984	0.003

Data are presented as M ± SD. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. the healthy control group. +++ $p < 0.001$ vs. the chronic asthma group. IL-4 = interleukin 4, NPY = Neuropeptide Y, ACTH = adrenocorticotropic hormone. HPA stress index was created as a composite index of cortisol, noradrenaline, adrenaline and ACTH using principal component analysis score (percentage of cumulative covariance: 84.467%).

Table 16. Correlations of psychological and biological stress variables with IL-4 level

	Whole sample		Healthy control		Chronic asthma		Acute asthma	
	r	*p*	r	*p*	r	*p*	r	*p*
NPY	0.19	0036	0.24	0.054	0.61	<0.001	0.39	0.18
ACTH	0.27	0.003	-0.14	0.26	-0.01	0.99	0.54	0.057
Noradrenaline	0.20	0.027	-0.31	0.010	0.15	0.32	0.39	0.18
Adrenaline	0.32	<0.001	-0.01	0.92	0.06	0.68	0.62	0.024
Cortisol ∗	-0.12	0.19	-0.12	0.35	-0.13	0.40	0.10	0.79
HPA Index	0.23	0.007	-0.26	0.034	0.10	0.50	0.73	0.001
Perceived stress	0.22	0.015	0.10	0.43	0.12	0.43	0.57	0.040

All data are adjusted for age. ∗ Adjusted for dosage of inhaled corticosteroid use (none, low dose (below 250 μg/day), and medium to high dose (equal to or above 250 μg/day)) in whole sample and chronic and acute asthma group. NPY = Neuropeptide Y, ACTH = adrenocorticotropic hormone. HPA stress index was created as a composite index of cortisol, noradrenaline, adrenaline and ACTH using principal component analysis score (percentage of cumulative covariance: 84.467%).

Table 17. Independent associations and mediational analyses of measures of perceived and HPA stress and NPY with IL-4 concentrations among patients with asthma

Independent variables	Dependent variable: IL-4 at baseline					Dependent variable: IL-4 at 1 year				
	R^2 Change	B	SE	β	*p*	R^2 Change	B	SE	β	*p*
Psychological stress models										
Model 1: Perceived stress	0.119	0.71	0.29	0.58	0.019	0.129	0.88	0.37	0.67	0.025
Model 2: NPY	0.479	28.82	4.31	0.75	<0.001	0.534	32.89	4.70	0.81	<0.001
Model 3: Perceived stress controlling for NPY	0.376	0.27	0.22	0.22	0.234 *	0.416	0.28	0.28	0.21	0.324 ‡
NPY controlling for perceived stress	0.109	26.50	4.25	0.69	<0.001	0.146	29.75	4.70	0.74	<0.001
Biological stress models										
Model 1: HPA index	0.085	3.49	1.43	0.29	0.018	0.052	2.75	1.72	0.23	0.115
Model 2: NPY	0.205	14.62	3.60	0.45	<0.001	0.381	22.89	4.25	0.62	<0.001
Model 3: HPA Index controlling for NPY	0.204	3.47	1.27	0.29	0.008	0.356	1.99	1.38	0.16	0.156
NPY controlling for HPA	0.084	14.58	3.43	0.45	<0.001	0.027	22.24	4.23	0.60	<0.001

* Sobel test: Z=2.134, p=0.033; ‡ Sobel test: z=2.143, p=0.032.

Analyses in the Psychological Stress model were controlled for base model variables: age, gender, ethnicity, housing type, smoking status, body mass index (BMI), asthma severity, and asthma control score and neuroticism.

R^2 change is change in model R^2 from base model upon addition of the candidate predictor variable. SE = standard error, NPY = Neuropeptide Y, ACTH = adrenocorticotropic hormone. HPA stress index was created as a composite index of cortisol, noradrenaline, adrenaline and ACTH using principal component analysis score (percentage of cumulative covariance: 84.467%).

CHAPTER 5 DISCUSSION

5.1 Study I: Prevalence of anxiety and depressive symptoms in adolescents with asthma: a meta-analysis and meta-regression

5.1.1 Higher prevalence of depressive symptoms among adolescents with asthma

There are approximately 300 million people worldwide suffering from asthma and annually 250,000 deaths attributed to the disease. [213] Among children under 15 years, asthma is the third-ranking cause of hospitalization. [214] By 2020, depressive symptoms will rank the second cause of disability globally. [215] Despite the abundance of literature reporting data on these two conditions, only eight studies met the inclusion criteria of this meta-analysis and this reflects the paucity of high-quality studies which examine the prevalence of depressive symptoms in adolescent asthma.

This study is, to our knowledge, the first meta-analysis aiming to estimate the aggregate prevalence of depressive and anxiety symptoms in adolescents with asthma compared with the healthy controls. The aggregate prevalence of depressive symptoms in adolescents with asthma was 27.0%, suggesting that at least one in four adolescents with asthma may experience depressive symptoms. Moreover, the aggregate prevalence of depressive symptoms was twice as high in adolescents with asthma as in the healthy controls. These findings suggest that asthma may be a risk factor for depressive symptoms in adolescents and this is hardly surprising as asthma affects daily functions that would require physical activity such as ambulation, mobility and school activities and psychosocial functioning. [95] However, the relationship between asthma and depression could be on the other way. The temporality of the association is required to be determined in large-scale prospective studies.

The current meta-regression demonstrated that proportions of Caucasian and smokers significantly increased the heterogeneity; whereas age, gender, and severity of asthma were not significant moderators. Smoking has long been considered a risk factor for anxiety and depressive symptoms. [216-218] Higher likelihood of frequent nocturnal coughing and breathing problems in smokers were reported in adolescent studies. [219, 220] Although the National Heart Lung and Blood Institute (NHLBI) recommends that persons with asthma should not smoke or be exposed to tobacco smoke in their environment, studies have found that the prevalence of smoking among those with asthma is the same as of those without asthma, or in some instances higher. [221-223] The higher prevalence of smoking can be explained by the self-medication hypothesis, [224] which describes the use of tobacco, drugs or other self-soothing forms of behavior to treat untreated and often undiagnosed mental distress, stress and anxiety. [225-227] This is often seen as a struggle to gain personal independence from conventional medical treatment. [228] Specifically, smoking may function as a compensatory means to modulate effects and treat distressful psychological states, whereby individuals with asthma may choose it to manage their depressive and anxiety symptoms and help them achieve emotional stability. [229, 230] As a potential confounder, stressful life events encountered by adolescents can independently affect the course of asthma while adolescents with asthma tend to cope with stressful life events by smoking. [231]

Consistent with other multi-ethnicity reports, [232-234] our analysis revealed the variability of prevalence of depressive symptoms in different ethnicities. Higher rates of depressive symptoms among Caucasians may reflect a tendency of them to express psychological distress compared with other ethnicities. [234] Furthermore, Caucasians generally tend to have higher socioeconomic status and more knowledge on psychological status and availability and accessibility of mental health services

utilization such as insurance, time, and transportation compared with the ethnic minorities. [235-237]

In this meta-analysis, severity of asthma was not a significant moderator in explaining the heterogeneity of calculating the OR of depressive symptoms among adolescents with asthma versus the healthy controls. This is consistent with studies indicating that anxiety and depressive symptoms in asthma patients may be independent of the severity of asthma. [238, 239] It may be the presence of asthma, but not the severity of the disease, which is associated with the comorbidities of psychological distress.

5.1.2 Higher prevalence of anxiety symptoms among adolescents with asthma

In this meta-analysis, it is noticeable that adolescents with asthma have higher risk (OR = 1.83) to develop anxiety symptoms and this finding is congruent with previous studies. [240-242] Several theories are proposed to explain the elevated risk for anxiety symptoms especially panic disorder among asthma patients. The biological and behavioral theories indicate that repetitive experience with hypoxia and hypercapnia in asthma attack may sensitize the amygdala and locus coeruleus and leads to overreaction to subsequent episodes of asthma attacks as a result of classical conditioning by pairing conditioned stimuli (i.e. sensation of breathlessness) and conditioned response (i.e. fear and anxiety). [228, 229] The cognitive theory suggests that the unpredictable and longitudinal experience with asthma attacks may generate fearful or catastrophic beliefs which provoke panic attacks and anticipatory anxiety. [228, 243] Children with anxiety disorders report more somatic symptom during inhalation of CO2-enriched air compared with children without anxiety disorder. [230, 244] Furthermore, anxiety and fear also cause changes in airway tone and precipitate an asthma attack. [245]

The impact of depressive and anxiety symptoms in patients with asthma is an important research area as it may be related to disease control and prognosis. [246] Alexander et al. (1972) and Weingarten et al. (1985) measured the effect of relaxation therapy on peak expiratory flow of asthmatic children and found effects favoring the treatment group compared with the control group. [247, 248] Brown et al. (2005) performed a randomized controlled trial by comparing the effects of citalopram, a selective serotonin reuptake inhibitor and placebo in asthma patients with depressive symptoms, [249] and found that antidepressant-treated patients required fewer oral corticosteroids than the placebo group. Furthermore, there is a lack of studies to draw conclusions regarding the utility of psychological approaches for improving asthma-related outcome. There is still a considerable need for good quality prospective studies to continue investigation into the causality and temporal relationship between asthma and depressive and anxiety symptoms and response to treatment.

The strength of this meta-analysis includes meta-regression and subgroup effect size analyses. Majority of the studies (seven out of eight) involved asthma patients from communities with relatively large sample size or population-based study designs, and this reflected the true prevalence in primary care which is largely free of clinical selection bias. Hence, our findings are more relevant to family physicians and public health policy makers in formulating strategies to reduce the burden of depressive and anxiety symptoms among adolescents with asthma in the community. [250, 251]

There are a few limitations in the current meta-analysis. The major limitation is that depressive and anxiety symptoms were assessed by various questionnaires and interviews but not clinical diagnosis

by doctors. Hence, the results of this study refer to the aggregate prevalence of depressive symptoms rather than depressive disorder. This study examined the cross-sectional data or the baseline data of cohort studies which cannot determine the causation or temporality of the association between psychological symptoms and adolescent asthma. Another limitation of the current study is the small number of studies involved, although the number of participants was reasonable (3,546 adolescent asthma patients and 24,884 healthy adolescent controls). We have considered only a small number of demographic and disease-related moderators in the meta-regression analysis due to the limited number of studies.

In conclusion, adolescents with asthma are in a higher risk to develop depression and anxiety. Family doctors, pediatricians and healthcare providers should formulate strategies to detect depressive and anxiety symptoms in adolescents with asthma and offer psychological interventions to reduce the burden of psychiatric comorbidity.

5.2 Study II: Asthma control, perceived stress, and quality of life in adolescents with asthma: a case-control study

In this study, we found higher levels of depression, panic attacks, total anxiety and total internalizing symptoms among adolescents with asthma when compared to the healthy controls. Our findings further support the association between adolescent asthma and psychiatric comorbidities that have been reported from previous studies. [252] The results from this study revealed no associations between social phobia, separation anxiety and adolescent asthma, whereas previous studies have reported elevated social phobia and separation anxiety in adolescents with asthma. [92, 253]

Among psychiatric comorbidities, the scores of panic symptoms and number of participants reaching clinical threshold of severe panic symptoms appeared to be highest, particularly for adolescents with poorly controlled asthma. This finding is consistent with previous studies. [254] Studies that carefully discriminated between symptoms of panic attacks and asthma attacks [255] have supported the observed excess comorbidity of panic attacks in asthma patients. [227] Patients with panic disorder were reported to have a three times higher risk of respiratory illnesses (such as asthma, bronchitis and emphysema) in comparison to patients with other psychiatric illnesses. [256] Nevertheless, further investigation is required before a conclusion of the relationship between asthma and panic attacks is drawn.

In this study, adolescents with asthma had not only more severe psychiatric comorbidities but also higher frequencies of socio-demographic variables that are known to be associated with both asthma and psychiatric comorbidities. Higher proportions of Malay and Indian ethnicities, past history of smoking, higher BMI and lower housing status were more common among adolescents with asthma, especially those with poorly controlled asthma as compared to the healthy controls. This finding is consistent with previous studies of the association of socio-demographic factors and smoking with asthma and depression. Previous reports have also shown that higher body weight and BMI were associated with relapse and more severe depressive and anxiety symptoms in asthma patients. [257]

The present study extends previous research findings by examining the major psychological factor of perceived stress, as a contributor to the observed excess of psychiatric comorbidities associated with asthma. Our findings indicate that adolescents with asthma had higher perceived stress than the

healthy controls. No significant differences in psychiatric comorbidities were observed after allowing for differences in this psychological factor. The results of a high level of perceived stress in patients with asthma in this study are consistent with the findings from previous studies. [253] In population-based studies, stressful life events were reported to be associated with an increased risk of asthma-related hospital admissions, [104] but daily hassles did not predict the onset of asthma. [150] In adults with asthma, [258] several types of stress were found not associated with asthma morbidity, but early traumatic life events predicted adult asthma onset. [259] A meta-analysis concluded that exposure to stressors alone does not increase the risk of allergic disorders, but only exposure to stressors which evoke negative cognition leads to an adverse impact on asthma patients. [61] These findings highlighted the importance of individual variation in perception of stress, and the importance of perceived stress in the course of asthma. Recurrent and unpredictable asthma attacks in poorly controlled asthma may cause patients to perceive high levels of stress, leading to psychiatric comorbidities.

Furthermore, the findings from this study showed that poor asthma control was associated with severe psychiatric comorbidities. This is consistent with previous findings that comorbid anxiety and depression among asthma patients were associated with more days of asthma symptoms, [95] poor treatment adherence and greater functional impairment. [202] The association between the level of asthma control and psychiatric symptoms was not significant after accounting for perceived stress, indicating the significance of perceived stress in the control of asthma symptoms and psychiatric comorbidities.

The present study has strengths and limitations. Asthma status was based on clinical diagnosis of

asthma by pediatricians, thus enhancing clinical accuracy and reducing symptom association bias. [156] We adjusted for important confounding by socio-demographic and smoking variables [202] in multivariate analyses. The healthy controls were age- and gender-matched adolescents residing in the same community with the patients, which ensures good comparability between the two groups. A major limitation of the study is that causal inferences cannot be determined from this cross-sectional study. Further longitudinal studies are required to examine the relationship between stress, asthma and psychiatric comorbidities. Because measures of psychiatric comorbidities and stress were based on self-reports, possible bias from differential recall and social desirability cannot be ruled out.

In conclusion, the presence of asthma and poor asthma control in adolescents are associated with psychiatric comorbidity, especially depression, panic attack, total anxiety and total internalizing symptoms. Perceived stress contributes to the observed excess psychiatric comorbidity associated with asthma, especially in poorly controlled asthma patients. Psychological therapy and support for asthmatics with high perceived stress should be integral in the management of asthma.

5.3 Study III: Asthma, psychological stress and psychiatric comorbidity: a population-based study in adult Singaporeans

Our study supports previous published findings of the strong association of asthma and other chronic physical conditions with psychiatric disorders and impaired QoL. About 14.2% of individuals with asthma were found to have a diagnosis of psychiatric disorder: 13.2% had depression; 6.6% had anxiety disorder.

Interestingly, in this study of Asian adults, asthma was found to be more evidently associated with depression than with anxiety disorder. Although some studies have reported higher rates of depressive symptoms in individuals with asthma, [207, 260, 261] there were negative findings as well. [18, 93] On the other hand, clinical and epidemiological studies typically involving children, adolescents and young adults have emphasized higher rates of anxiety disorders such as panic attacks in asthma cases, when compared to their non-asthmatic counterparts or those who had other chronic physical conditions. [18, 262-264] In many studies using symptom scales, it may be difficult to distinguish perceived symptoms that could be misinterpreted as both asthma/dyspnea and anxiety symptoms/panic disorder. This study used a standardized and well-validated diagnostic interview to assess a range of common psychiatric disorders and the results showed notably lower prevalence of GAD. Furthermore, in this community-based study, the results are less likely to be influenced by selection bias associated with help-seeking by anxious patients in studies in clinical settings.

We observed in this study that while psychiatric disorders were nearly equally more common in individuals with asthma and those with other chronic physical conditions, excessive stressful life events were four times more frequent in respondents with asthma compared to those with other chronic physical conditions. As a known risk factor for psychiatric disorders, an excess of stressful life events appeared to mediate the observed comorbidity of psychiatric disorders in asthma, more than in other chronic physical conditions in this cross-sectional study. Extensive literature [12, 265] has described the stress and anxiety provoked by breathing difficulties/dyspnea, including muddled thoughts, heightened emotions, extreme fear and panic and decreased physical energy. Recent studies indicate that patients with asthma attacks and anaphylaxis had an excess of PTSD symptoms and coexisting psychiatric disorders, namely, somatic problems, anxiety, social dysfunction and

depression. [266, 267] Although empirical evidence is still limited, there are also reports of an increase in asthma exacerbations in children immediately following a stressful life event. [268] There is also intriguing evidence suggesting that negative emotions such as sad mood or anxiety produce respiratory effects that are consistent with airway instability or asthma exacerbations. [269] This relationship is biologically plausible in terms of the down-regulation of the normal stress response pathways in the HPA axis and the SAM system, which in turn alters cytokine profiles favoring allergic inflammation. [270] Further research is required on this mutual relationship between asthma and response to stressors and coexisting psychiatric disorders.

Our finding of worse QoL among individuals with asthma compared to those without chronic physical conditions was expected and is well established. [271, 272] Among individuals with asthma, studies also suggest that the co-occurrence of psychiatric disorders significantly affects performance of daily activities and use of health care services. [273, 274] In the present study, we found that QoL was more impaired in individuals with asthma compared to individuals with other physical conditions as well; furthermore, stressful life events made large contributions (R^2 = 0.16) to the observed differences in impaired QoL, whereas diagnoses of depression and anxiety disorders contributed less (model R^2 = 0.03 to 0.11). This suggests that in community-based settings, addressing stress and psychological disturbance beyond the clinical diagnosis of mental disorders is likely to make considerable impact in alleviating poor QoL in individuals with asthma.

The strengths of the present study include the population-based design and large sample size. The results from this general population-based study are largely free of clinical selection bias, and

controlled for important confounding by demographic and psychosocial variables in the analysis. While most studies have investigated psychological symptoms, the present study used a standardized and well-validated diagnostic interview to assess a range of common mental disorders. There are several limitations in this study. The case definition for asthma based on self-report is not ideal. However, studies have indicated that patients' self-reports of most chronic diseases which are largely symptoms-based diagnoses such as asthma, are generally accurate. [275, 276] To minimize ascertainment errors, we asked subjects to allow inspection of their asthma medications. Misclassification errors were also likely to result from unrecognized or untreated illnesses, or screen-negative cases of psychiatric disorders. Missing values on LTEQ for a small number of items and study participants may also affect the accuracy of our results. Finally, firm causal inferences cannot be made from the cross-sectional findings in this study.

In conclusion, coexisting psychiatric disorders are more likely to be observed in individuals with asthma or other chronic physical conditions, compared to those without chronic physical conditions. However, an excess of stressful life events is more common in individuals with asthma, and mediates the association of asthma and psychiatric disorder and contributes to impaired QoL. These relationships should be further investigated in future longitudinal studies.

5.4 Study IV: The impact of stressful life events on quality of life in the elderly with airway obstruction

The principal finding in this study indicated that stressful life events were associated with depressive symptoms and poor quality of life in participants with or without airway obstruction (main effects),

but showed a significantly stronger association among individuals with airway obstruction than those without airway obstruction (interaction), suggesting a disproportionately greater detrimental effect of stressful life events on airway obstruction. To our knowledge, no other studies have reported demonstrating this relationship.

It should be noted that participants with airway obstruction actually did not report greater frequency of occurrence or perceived stress score of non-illness related life events than non- airway obstructive participants. Instead, individuals with airway obstruction appeared to experience the same number of non-illness related life events and perceived them to be equally stressful as their non- airway obstructive counterparts, yet showed disproportionately greater psychological distress and poorer QoL. These results suggest that individuals with airway obstruction may be more vulnerable to the adverse impact of stressful life events than non- airway obstructive individuals. This may be due to different perception and appraisement of stressful life events, or poorer coping skills and fewer social and economic resources in individuals with airway obstruction, or both. We did not measure cognitive appraisal, coping resources and social support to explore these hypotheses directly, and this is a limitation of our study.

There are few studies that have investigated the relationship between cognitive appraisal of stressful life events, coping strategies and psychological distress in individuals with airway obstruction. A study by Andrenas et al. [64] assessed how hospitalized patients with acutely exacerbated COPD appraised and coped with a recent stressful life events and their level of psychological distress. They reported that half of the respondents tended to perceive their stressful life events as representing a

threat, 26% as harmful, 7.6% as a loss, 4.3% as a challenge, and 11% characterized the stressful event in some other ways. However, the authors failed to find any association of psychological distress with types of stressful life events, stress intensity, primary or secondary appraisal, or number of coping strategies used. Only problem-solving coping strategies were inversely correlated to psychological distress. This suggests that poor coping skills may be the principal psychological problem among COPD patients that contribute to their psychological distress and poor QoL. Further studies are required to investigate the psychological characteristics of individuals with airway obstruction.

Our secondary finding of the main effects of airway obstruction on FEV_1 was expected and thus not surprising. However, the association of airway obstruction with more frequent cognitive problems was interesting, although the results for MMSE score were not significant after adjustment in two-way ANCOVA, possibly due to sample size limitation. These results are consistent with clinical and population studies that indicate the impact of chronic airway obstruction on deficits in abstract reasoning, [277] complex visual motor process, [278] verbal learning, [279] language, [280] attention, [281] information processing speed, [281-283] verbal learning and memory [282, 283].

The present study has strengths and limitations. The case definition for airway obstruction is accurately based on post-bronchial dilatation spirometric measures of chronic airflow obstruction according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommended criteria. The measure of life event stress is modified to exclude illness-related stress from chronic diseases in this older population. The limitations of questionnaire measurement of stressful life

events and cross-sectional study design hinder precise assessment of stressful life events and interpretation of a causal relationship between stress and health-related functional outcomes.

Studies [8-10] have reported that mental health status, including anxiety and depressive symptoms, are better predictors of chronic airway obstruction-related QoL than pulmonary function. The present study supports this observation and further indicates that stressful life events have a starkly detrimental effect on mental health and QoL in individuals with airway obstruction. More studies of the effects of stress management and coping strategy in psychological interventions in airway obstruction should be investigated in randomized controlled clinical trials. It is increasingly being recognized that the identification of mood and anxiety disorders, and psychological and psychosocial interventions to improve mood and reduce anxiety are important for improving patient centered outcomes in chronic airway obstructive patients. However, in published clinical guidelines, where the initial step care management by practitioners in primary care and general hospital settings includes low-intensity psychosocial interventions for patients with persistent subthreshold depressive symptoms or mild to moderate depression, there appears to be little attention given to identifying stressful life event(s) and supporting chronic airway obstructive patients experiencing stressful life events to prevent the onset of mood and anxiety disorders. In particular, group-based peer support, individual guided self-help based on cognitive behavioral therapy (CBT) principles or computerised CBT to reduce patients' vulnerability to stress may usefully include objective cognitive appraisal of stress, problem-solving coping skills, and relaxation therapy to help support chronic airway obstructive patients experiencing stressful life events.

In conclusion, this study found that stressful life events were associated with more depressive symptoms and worse QoL in individuals with airway obstruction, much more than in those without airway obstruction. Further studies should explore the role of cognitive appraisal of stress, coping resources and psycho-social support in this relationship.

5.5 Study V: Stress, neuropeptide Y, and young adult asthma: a follow-up study

Psychological stress and asthma In the present study, we found that although exposure to stressful life events did not differ among groups, asthma patients, especially those with acute asthma, experienced higher levels of perceived stress than their healthy control counterparts. The high levels of perceived stress in asthma patients were corroborated by higher levels of ACTH, noradrenaline, adrenaline, and the HPA index, and lower levels of NPY and cortisol, compared with healthy controls. Furthermore, perceived stress predicted elevated levels of IL-4, which is important in the physiopathology of Th1/ Th2 imbalance [284] in asthma. This is consistent with previous research documenting the strong link between psychological stress and asthma. Studies suggest that traumatic life events in early childhood predict subsequent asthma onset [259] and stressful life events such as a death in the family and mourning have been shown to trigger the first attack of asthma, [63] an increase in the risk of new onset asthma, [59] or asthma-related hospital admissions. [104] Perceived stress is reported to be associated with increased asthma incidence, daily medication intake and first-time hospitalization risk. [55, 61]

Psychological stress, HPA activation and cortisol Although a disruption in neuroendocrine-immune system communication involving the HPA axis is known to play a major role in the adverse health

effects of psychological stress, [74] the specific mechanism underlying the association between psychological stress and asthma is not well elucidated. Our results showed that among the patients with asthma, the elevated peripheral levels of ACTH, noradrenaline and adrenaline, and HPA index indicate stress-induced activation of the HPA axis and adrenal medulla in asthma. Studies have shown that bronchoconstriction is not a stimulus for sympatho-adrenal activation, [285] hence the observed activated HPA stress response was most probably due to psychological stress rather than airway disorder. The concurrent low levels of cortisol observed in asthma patients is consistent with studies which observed a marked rise in ACTH concentrations with no concomitant increments or markedly diminished cortisol concentrations, [286, 287] indicating a dampened cortisol response related to a disturbance in stress-induced HPA activation. Chronic stress is thought to result in adaptive mechanisms of glucocorticoid receptors in the form of increased negative feedback sensitivity, hence leading to lower cortisol secretion. [288]

Psychological stress and NPY Studies of U.S. army soldiers participating in survival training indicate that under normal conditions, acute stress elicits NPY release and that this release is positively associated with cortisol and NE release; greater levels of NPY release are associated with less psychological distress, suggesting that NPY confers anxiolytic activity. [289] The anxiolytic effect of NPY under physiological conditions is supported by animal studies in which intracerebroventricular administration of NPY in rats reduced social (intruder) stress as measured by significant decrease in blood pressure, heart rate, and activity. [290] On the other hand, under chronic conditions of prolonged stress, combat veterans with PTSD compared to healthy individuals are documented to exhibit both lower NPY and cortisol levels in plasma, and evidence of increased anxiety and sympathetic system arousal. [138] Lower plasma NPY levels were associated with greater degrees of combat exposure,

greater symptoms of PTSD, and levels of norepinephrine and systolic blood pressure induced by a noradrenergic α2-antagonist, yohimbine, suggesting a dampened NPY response. [291, 292] Our results of lower levels of NPY and cortisol and higher levels of ACTH, adrenaline and noradrenaline in patients with chronic asthma, both non-exacerbated and acutely exacerbated, than the healthy controls are in concordance with these prior findings supporting the effect of chronic stress exposure in blunting NPY responsiveness [293] with associated dysregulation of the sympathetic nervous system.

Asthma and IL-4 The pathogenesis of asthma is widely believed to involve airway inflammatory response mediated by CD4+ T cells with a bias toward Th2 responses in the balance between Th1 and Th2 responses. [294] IL-4 is a key Th2 cytokine and an essential cofactor for IgE production that triggers a humoral immune response toward up-regulation of IgE, a prototypic feature of allergies. [284, 295] The release of IL-4 is thus a key marker of allergic inflammation that correlates well with asthma severity. [296, 297] Higher levels of IL-4 are reported in children with both controlled and uncontrolled asthma than healthy controls. [298, 299] Furthermore, increased levels of IL-4 are reported to be associated with acute stress events among children with asthma and chronic family stress. [300] Our observation of higher circulating levels of IL-4 among asthma patients especially those with acute exacerbations are in agreement with previous reports. [298, 299]

Psychological stress, IL-4 and NPY mediation in asthma Stress is believed to alter immune response features of asthma which involve a bias away from cellular (Th1) immunity and towards humoral (Th2) immunity, typically manifested by the over-expression of Th2 cytokines and manifested in asthma exacerbations. [301, 302] A prior study has found that children with asthma who had higher levels

of chronic stress showed increased production of IL-4 in response to acute stress. [300] We replicated this finding among the patient groups with non-exacerbated and acutely exacerbated chronic asthma in this study. We further found that perceived stress among asthma patients was a significant predictor of IL-4 levels measured both concurrently at baseline and one year later; suggesting that perceived stress has a persistent effect in heightening Th2 immune and inflammatory responses in asthma. We further extend this finding by providing a plausible biological mechanism for the influence of perceived stress on the immune-inflammatory profile in asthma. Our data strongly suggests that plasma levels of NPY mediated the association of high perceived stress levels with the over-expression of IL-4 in asthma.

We also explored the HPA index as an integrated measure of the body's systemic HPA and SAM-related response to a totality of internal biological stressors, and its effect on the levels of Th2-associated IL-4 expression in asthma. In like manner, the results indicated that a heightened level of HPA activity was associated with elevated levels of IL-4 in asthma. In keeping with its temporal measurement of acute stress response, the HPA index did not predict IL-4 levels at one-year follow up. NPY level did not appear to mediate this relationship. Our data suggest that NPY concentration is a steady state measure of neuroendocrine activity, and consistently and independently predicted a long-lasting Th2-associated hyper-immune and inflammatory response in asthma.

NPY and IL-4 in asthma The relationship between NPY and IL-4 in asthma is not well understood. Biological studies have yielded controversial and ambiguous findings, [303, 304] and reports of human studies are scarce. NPY activation of NPY-Y1 on antigen-presenting cells (APCs) is essential for T

cell priming, but paradoxically it acts as a negative regulator for T cells and modulates T cell hyper-responsiveness, thus suggesting a bimodal role in APCs and T cells in the immune system. [303, 305, 306] Studies in mice models of asthma suggest that NPY was elevated at the late (chronic) stage of the airway inflammatory process, and was inversely correlated with the level of IL-4, suggesting an effect in attenuating Th2 cytokine production and release. [307] It remains unclear whether the increase in NPY is a defensive or compensatory mechanism to modulate the effects of inflammatory cytokines. A limited number of human studies have yielded equivocal findings. Among young asthma patients, plasma concentrations of NPY measured before and during bronchoconstriction induced by histamine or allergens remained unchanged up to 30 minutes after bronchoconstriction. [285] Another study showed that children with mild asthma and healthy controls reportedly did not show any differences in NPY levels. [302] A study of elderly patients with acute asthma attacks in an emergency medicine ward reported elevated plasma concentration of NPY, but notably the authors concluded that circulating NPY under these conditions more likely had a nervous rather than adrenal origin. [301] Our study showed that NPY levels, consistent with a dampened response associated with chronic psychological stress, were lower among acutely exacerbated and non-exacerbated asthma patients, compared to healthy controls. However, among asthma patients, an elevated level of NPY was found to be associated with a persistent elevation of IL-4 which is a prominent marker of Th2-biased immune and inflammatory profiles. This paradoxical association may possibly reflect increased NPY-ergic activity in response to high IL-4 in asthma. Given the role of NPY in the neural control of immune responses, increased secretions of NPY in the late stage of the airway inflammatory process normally acts to attenuate Th2 cytokines production and release in asthma. Further studies are needed to elucidate these relationships.

Our findings are important since they add new insights into the psychobiological mechanisms underlying the relationships between psychological stress and asthma. However, there are limitations to this study. The cross-sectional design of the study makes it difficult to interpret the temporal causal relationship especially between NPY and IL-4. The present study used peripheral and not central measures of NPY to assess neuroendocrine responses to stress. Studies have shown that cerebrospinal fluid (CSF) levels of NPY are approximately three times higher than plasma levels of NPY, and the correlation between plasma NPY and CSF NPY is weak ($r = 0.29$). [308] Although evidence suggests strikingly similar response patterns of central and peripheral NPY to stress, [309] future brain imaging research using specific NPY-receptor ligands would be helpful to address the relationship between central release of NPY and psychological and immune functions. Another limitation is that the atopic status of the asthma patients in the study was not characterized. The relatively small sample size from this study may have limited the power of the study to detect significant differences among subgroups and to generalize findings.

CHAPTER 6 SUMMARY AND CONCLUSION

In a series of studies (Study I to Study V), this thesis investigated the contribution of psychological stress to the psychiatric comorbidities and functioning impairment observed in obstructive airway diseases in adolescent, adult and elderly individuals. Among young adults who are free from comorbidity with other respiratory diseases, we assessed the potential mediating role of NPY in the association between perceived stress and asthma.

Results of the present thesis supported our hypothesis of a pivotal role of psychological stress, assessed in this study as stressful life events and perceived stress, in the close association of psychological symptoms and obstructive airway diseases. Asthma was associated with higher levels of depression, panic attacks, total anxiety and total internalizing symptoms in adolescents, and a higher prevalence of any psychiatric disorder, major depressive disorder and generalized anxiety disorder among adults. Participants with asthma, especially those with poorly controlled asthma, reported significantly lower quality of life scores. Meanwhile, higher levels of perceived stress were found in adolescents and young adults with asthma compared to the healthy controls, and among poorly controlled asthma patients and patients with acute exacerbations. The mean stressful life event score in adults with asthma was approximately 3 times as high as in adults with other chronic medical conditions and 5 times as high as in the healthy controls. Furthermore, stressful life events and perceived stress contributed, to a great extent in hierarchical regression models, to the psychiatric comorbidity and impaired quality of life among adolescents and adults with asthma. In the elderly, stressful life events were found to be associated with more depressive symptoms and worse physical and mental functioning in respondents with airway obstruction than in those without airway obstruction.

The neuroendocrine and immune physiology underlying the association of psychological stress and asthma is not well studied in humans. The present thesis investigated the association of measures of psychological and biological stress with Th2 expression of IL-4 and the mediating role of NPY in this association among young adults with acute and stable chronic asthma and their age- and gender-matched controls. Asthma was associated with higher levels of psychological stress, corroborated by elevated levels of HPA-related levels of ACTH, NA, A, and depressed levels of cortisol and NPY, reflecting blunted adaptive responses to chronic stress. Higher levels of perceived stress were further shown to be associated with increased levels of IL-4 among the asthma patients. The precipitating effect of perceived stress on IL-4 was mediated by the levels of NPY, and this relationship might be lasting by observing their associations with a repeat measure of IL-4 levels at follow up one year later. The HPA index measure of transient biological stress, independent of NPY, was a significant predictor of IL-4 at baseline but not at one-year follow up. These findings confirm our hypothesis of a pivotal role of NPY in the association between psychological stress and asthma. More studies are required to elucidate its immune-modulatory role in asthma.

The current study investigated the relationship between psychological (stress, depression and anxiety), immunological (inflammatory cytokines) and neuro-endocrine and humoral (NPY, ACTH, cortisol, adrenaline, noradrenaline, HPA stress index) status of individuals with airway obstruction (well controlled and poorly controlled, with acute exacerbation and in the quiescent state) and healthy controls and results emphasized the role of stress in the severity, symptom control, psychological health and quality of life of individuals with obstructive airway diseases, which may be mediated partly by a stress-related neurotransmitter named NPY. Research like this study from a

psycho-neuro-immunological perspective might be helpful to enhance, to some extent, the understanding of the link between stress and airway obstruction and the inter-individual variation in response to stress and airway obstruction in adolescent, adult and elderly individuals. In view of our findings, treatment and control of asthma and airway obstruction should seriously consider the excess rates of comorbid depression and anxiety, which is particularly high in patients with acute exacerbations or poorly controlled asthma. High levels of perceived stress are key vulnerability factors for comorbid psychiatric comorbidity, and it is advisable to screen patients for psychological treatment. CBT can target cognitive bias such as the tendency to overestimate stress and negative experience. Relaxation therapy, as well as peer group support among patients with airway obstruction, may be included to reduce the risks of panic attacks, negative emotions and stress levels. The significance of NPY as a "resilience factor" in modulating stress exposure and asthma morbidity supports the recently proposed use of NPY agonists [310] in treating stress-exacerbated asthma which represents a psychosomatic approach in pharmacological therapy of asthma and other psychosomatic disorders. To confirm the potential benefits of NPY agonists in stress modulation, randomized controlled trials targeting the high-risk population (for example, those with low NPY and high perceived stress) could be performed in the future.

The findings from this study should be interpreted with caution because of multiple limitations. Potential bias from differential recall and social desirability could not be ruled out because quite a number of measurements in this study (such as anxiety and depression, QoL and perceived stress) were based on self-reported questionnaires. Given the time constraint of a PhD thesis, our studies had either cross-sectional (Study II-Study IV) or relatively short follow-up (Study V) study designs. Therefore, reference of a temporal relationship, though desirable was not feasible. In Study V which

examined the role of NPY in resilience to stress, the interval between baseline and follow-up assessment was 1.0 year in median and might not be long enough to reveal significant associations. The follow-up of the SLAS (Study IV) is ongoing and future data may provide more evidence regarding the temporality.

Although emergent evidence supports the association of stress and airway obstruction, the underlying mechanism is poorly understood. Stress response is complicated which involves the interaction of multiple psychobiological, genetic, and environmental factors from multiple systems. We believe that the work presented in this thesis could add a little more to the growing knowledge of the psychological and neuroendocrine response pattern to stress in individuals with airway obstructive diseases. We postulate that future multidisciplinary studies using neurochemical, neuroimaging, and genetic approaches may be helpful for elucidating a full picture of the genotype, phenotype, and psychobiological responses to stress. Since its discovery 30 years ago, the role of NPY in multiple pathophysiological processes such as cardiovascular diseases, stress, and obesity has drawn attention from researchers, and preliminary findings have been accumulating. Hopefully, related research could clarify the role of NPY in stress which is involved in a neurobiological response promoting resilience to psychological stress in the near future. In the long run, substantial progress in research findings is urgently needed to enhance the capabilities of human beings to predict, prevent, and treat stress-related psychopathology which has been growing in the 21st century.

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APPENDIX

Asthma Control Questionnaire

Please circle **ONE** answer for each of the five questions below. Be sure to review your results with your healthcare professional.

1. In the past four weeks, how much of the time did your asthma keep you from getting as much done at work or at home?	5). none of the time	4). a little of the time	3). some of the time	2). most of the time	1). all of the time
2. During the past four weeks, how often have you had shortness of breath?	5). not at all	4). once or twice a week	3). 3 to 6 times a week	2). once a day	1). more than once a day
3. During the past four weeks, how often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightnss or pain) wake you up at night, or earlier than usual in the morning?	5). not at all	4). once or Twice	3). once a week	2). 2 to 3 nights a week	1). 4 or more nights a week
4. During the past four weeks, how often have you used your rescue inhaler or nebulizer medication (such as albuterol)?	5). not at all	4). once a week or less	3). a few times a week	2). 1 or 2 times per day	1). 3 or more times per day
5. How would you rate your asthma control during the past four weeks?	5).completely controlled	4). well controlled	3). somewhat controlled	2). poorly controlled	1). not controlled at all

Revised Child Anxiety and Depression Scale

Please put a circle around the word that shows how often each of these things happen to you. There are no right or wrong answers.

	Never	Some times	Often	Always
1. I worry about things	0	1	2	3
2. I feel sad or empty	0	1	2	3
3. When I Have a problem, I get a dummy feeling in my stomach	0	1	2	3
4. I worry when I think I have done poorly at something	0	1	2	3
5. I would feel afraid of being on my own at home	0	1	2	3
6. Nothing is much fun anymore	0	1	2	3
7. I feel scared when I have to take a test	0	1	2	3
8. I feel worried when I think someone is angry at me	0	1	2	3
9. I worry about being away from my parents	0	1	2	3
10.I get bothered by sad or silly thoughts or pictures in my mind	0	1	2	3
11.I have trouble sleeping	0	1	2	3
12.I worry that I will do badly at my school work	0	1	2	3
13.I worry that something awful will happen to someone in my family	0	1	2	3
14.I suddenly feel as if I can't breathe when there is no reason for this	0	1	2	3
15.I have problems with my appetite	0	1	2	3
16.I have to keep checking that I have done things right (like the switch is off, or the door is locked)	0	1	2	3
17.I feel scared if I have to sleep on my own	0	1	2	3
18. I have trouble going to school in the mornings because I feel nervous or afraid	0	1	2	3
19.I have no energy for things	0	1	2	3
20.I worry I might look foolish	0	1	2	3
21.I am tired a lot	0	1	2	3
22.I worry that bad things will happen to me	0	1	2	3
23.I can't seem to get bad or silly thoughts out of my head	0	1	2	3

24.When I have a problem, my heart beats really fast	0	1	2	3
25.I cannot think clearly	0	1	2	3
26. I suddenly start to tremble or shake when there is no reason for this	0	1	2	3
27.I worry that something bad will happen to me	0	1	2	3
28.When I have a problem, I feel shaky	0	1	2	3
	Never	Sometimes	Often	Always
29.I feel worthless	0	1	2	3
30.I worry about making mistakes	0	1	2	3
31.I have to think of special thoughts (like numbers or words) to stop bad things from happening	0	1	2	3
32.I worry that other people think of me	0	1	2	3
33.I am afraid of being in crowded places (like shopping centers, the movies, buses, usy playgrounds)	0	1	2	3
34.All of a sudden I feel really scared for no reason at all	0	1	2	3
35.I worry about what is going to happen	0	1	2	3
36.I suddenly become dizzy or faint when there is no reason for this	0	1	2	3
37.I think about death	0	1	2	3
38.I feel afraid if I have to talk in front of my class	0	1	2	3
39.My heart suddenly starts to beat too quickly for no reason	0	1	2	3
40.I feel like I don’t want to move	0	1	2	3
41.I worry that I will suddenly get a scared feeling when there is nothing to be afraid of	0	1	2	3
42.I have to do some things over and over again (like washing my hands, cleaning or putting things in a certain order)	0	1	2	3
43.I feel afraid that I will make a fool of myself in front of people	0	1	2	3
44.I have to do some things in just the right way to stop bad things from happening	0	1	2	3
45.I worry when I go to bed at night	0	1	2	3
46.I would feel scared if I had to stay away from home overnight	0	1	2	3
47.I feel restless	0	1	2	3

Hospital Anxiety and Depression Scale (HADS)

Please circle one answer that suits you most from the four given for each question. Give an immediate response and do not thinking too long about the answers.

A	**I feel tense or 'wound up':**	
	Most of the time	3
	A lot of the time	2
	From time to time, occasionally	1
	Not at all	0

D	**I still enjoy the things I used to enjoy:**	
	Definitely as much	0
	Not quite so much	1
	Only a little	2
	Hardly at all	3

A	**I get a sort of frightened feeling as if something awful is about to happen:**	
	Very definitely and quite badly	3
	Yes, but not too badly	2
	A little, but it doesn't worry me	1
	Not at all	0

D	**I can laugh and see the funny side of things:**	
	As much as I always could	0
	Not quite so much now	1
	Definitely not so much now	2
	Not at all	3

A	**Worrying thoughts go through my mind:**	

	A great deal of the time	3
	A lot of the time	2
	From time to time, but not too often	1
	Only occasionally	0

D	**I feel cheerful:**	
	Not at all	3
	Not often	2
	Sometimes	1
	Most of the time	0

A	**I can sit at ease and feel relaxed:**	
	Definitely	0
	Usually	1
	Not Often	2
	Not at all	3

D	**I feel as if I am slowed down:**	
	Nearly all the time	3
	Very often	2
	Sometimes	1
	Not at all	0

A	**I get a sort of frightened feeling like 'butterflies' in the stomach:**	
	Not at all	0
	Occasionally	1
	Quite Often	2
	Very Often	3

D	**I have lost interest in my appearance:**	

	Definitely	3
	I don't take as much care as should	2
	I may not take quite as much care	1
	I take just as much care as ever	0

A	**I feel restless as I have to be on the move:**	
	Very much indeed	3
	Quite a lot	2
	Not very much	1
	Not at all	0

D	**I look forward with enjoyment to things:**	
	As much as I ever did	0
	Rather less than I used to	1
	Definitely less than I used to	2
	Hardly at all	3

A	**I get sudden feelings of panic:**	
	Very often indeed	3
	Quite often	2
	Not very often	1
	Not at all	0

D	**I can enjoy a good book or radio or TV program:**	
	Often	0
	Sometimes	1
	Not often	2
	Very seldom	3

The Holmes-Rahe Social Readjustment Ratings Scale (for children)

Please put a circle around the word that happened to you **during the last year** in the following list.

	Yes	No
1.Getting married	1	0
2.Unwed pregnancy	1	0
3.Death of parent	1	0
4.Acquiring a visible deformity	1	0
5.Divorce of parents	1	0
6.Fathering an unwed pregnancy	1	0
7.Becoming involved with drugs or alcohol	1	0
8.Jail sentence of parent for over one year	1	0
9.Marital separation of parents	1	0
10.Death of a brother or sister	1	0
11.Change in acceptance by peers	1	0
12.Pregnancy of unwed sister	1	0
13.Discovery of being an adopted child	1	0
14.Marriage of parent to step-parent	1	0
15.Death of a close friend	1	0
16.Having a visible congenital deformity	1	0
17.Serious illness requiring hospitalization	1	0
18.Failure of a grade in school	1	0
19.Not making an extracurricular activity	1	0
20.Hospitalization of a parent	1	0
21.Jail sentence of parent for over 30 days	1	0
22.Breaking up with boyfriend or girlfriend	1	0
23.Beginning to date	1	0

24.Suspension from school	1	0
25.Birth of a brother or sister	1	0
26.Increase in arguments between parents	1	0
27.Loss of job by parent	1	0
28.Outstanding personal achievement	1	0
29.Change in parent's financial status	1	0
30.Accepted at a college of your choice	1	0
31.Being a senior in high school	1	0
32.Hospitalization of a sibling	1	0
33.Increased absence of parent from home	1	0
34.Brother or sister leaving home	1	0
35.Addition of third adult to family	1	0
36.Becommg a full fledged member of a church	1	0
37.Decrease in arguments between parents	1	0
38.Decrease in arguments with parents	1	0
39.Mother or father beginning work	1	0

The Holmes-Rahe Social Readjustment Ratings Scale

Please put a circle around the word that happened to you **during the last year** in the following list.

	Yes	No
1. Death of a Spouse	1	0
2. Divorce	1	0
3. Marital Separation	1	0
4. Imprisonment	1	0
5. Death of a Close Family Member	1	0
6. Personal Injury or Illness	1	0
7. Marriage	1	0
8. Dismissal from Work	1	0
9. Marital Reconciliation	1	0
10. Retirement	1	0
11. Change in Health of Family Member	1	0
12. Pregnancy	1	0
13. Sexual Difficulties	1	0
14. Gain a New Family Member	1	0
15. Business Readjustment	1	0
16. Change in Financial State	1	0
17. Change in Frequency of Arguments	1	0
18. Major Mortgage	1	0
19. Fore closure of Mortgage or Loan	1	0
20. Change in Responsibilities at Work	1	0
21. Child Leaving Home	1	0
22. Trouble with In-Laws	1	0
23. Outstanding Personal Achievement	1	0

24. Spouse Starts or Stop Work	1	0
25. Begin or End School	1	0
26. Change in Living Conditions	1	0
27. Revision of Personal Habits	1	0
28. Trouble with Boss	1	0
29. Change in Working Hours or Conditions	1	0
30. Change in Residence	1	0
31. Change in Schools	1	0
32. Change in Recreation	1	0
33. Change in Church Activities	1	0
34. Change in Social Activities	1	0
35. Minor Mortgage or Loan	1	0
36. Change in Sleeping Habits	1	0
37. Change in Number of Family Reunions	1	0
38. Change in Eating Habits	1	0
39. Vacation	1	0
40. Christmas	1	0
41. Minor Violation of Law	1	0

Perceived Stress Scale (PSS)

These questions ask you about your feelings, thoughts and activities **during the last month, including today**.

In the last month, how often have you:

	Never	Almost Never	Sometimes	Fairly Often	Very Often
1. Been upset because of something that happened unexpectedly?	0	1	2	3	4
2. Felt that you were unable to control important things in your life?	0	1	2	3	4
3. Felt nervous and "stressed"?	0	1	2	3	4
4. Felt confident about your ability to handle your personal problems?	0	1	2	3	4
5. Felt that things were going your way?	0	1	2	3	4
6. Found that you could not cope with all things you had to do?	0	1	2	3	4
7. Been able to control irritations in your life?	0	1	2	3	4
8. Felt that you were on top of things?	0	1	2	3	4
9. Been angered because of things that happened that were out of your control?	0	1	2	3	4
10. Felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Pediatric Asthma Quality of Life Questionnaire

We want you to tell us how much you have been bothered doing these things **during the last week because of your asthma.**

Circle in the box that best describes how bothered you have been.

HOW BOTHERED HAVE YOU BEEN DURING THE LAST WEEK?								
	Extremely Bothered	Very Bothered	Quite Bothered	Somewhat Bothered	Bothered A Bit	Hardly Bothered	Not Bothered	Activity Not Done
1.Playing at recess	1	2	3	4	5	6	7	0
2.Running	1	2	3	4	5	6	7	0
3.Sleeping	1	2	3	4	5	6	7	0
4.Coughing	1	2	3	4	5	6	7	0

IN GENERAL, HOW OFTEN DURING THE LAST WEEK DID YOU:							
	All of the Time	Most of the time	Quite Often	Some of the Time	Once in a While	Hardly any of the Time	None of Time
5. Feel FRUSTRATED because of your asthma?	1	2	3	4	5	6	7
6. Feel TIRED because of your asthma?	1	2	3	4	5	6	7
7. Feel WORRIED, CONCERNED OR TROUBLED because of your asthma?	1	2	3	4	5	6	7

HOW BOTHERED HAVE YOU BEEN DURING THE LAST WEEK BY?							
	Extremely Bothered	Very Bothered	Quite Bothered	Somewhat Bothered	Bothered a Bit	Hardly Bothered	Not Bothered
8. Asthma Attacks?	1	2	3	4	5	6	7

IN GENERAL, HOW OFTEN DURING THE LAST WEEK DID YOU:							
	All of the Time	Most of the Time	Quite Often	Some of the Time	Once in a While	Hardly Any of the Time	None of Time
9. Feel ANGRY because of your asthma?	1	2	3	4	5	6	7

HOW BOTHERED HAVE YOU BEEN DURING THE LAST WEEK BY?							
	Extremely Bothered	Very Bothered	Quite Bothered	Somewhat Bothered	Bothered a Bit	Hardly Bothered	Not Bothered
10. Wheezing	1	2	3	4	5	6	7
IN GENERAL, HOW OFTEN DURING THE LAST WEEK DID YOU:							
	All of the Time	Most of the Time	Quite Often	Some of the Time	Once in a While	Hardly Any of the Time	None of Time
11. Feel IRRITABLE / Grumpy because of your asthma?	1	2	3	4	5	6	7
HOW BOTHERED HAVE YOU BEEN DURING THE LAST WEEK BY?							
	Extremely Bothered	Very Bothered	Quite Bothered	Somewhat Bothered	Bothered a Bit	Hardly Bothered	Not Bothered
12. Tightness in your chest	1	2	3	4	5	6	7
IN GENERAL, HOW OFTEN DURING THE LAST WEEK DID YOU:							
	All of the Time	Most of the Time	Quite Often	Some of the Time	Once in a While	Hardly Any of the Time	None of Time
13. Feel different or left out because of your asthma?	1	2	3	4	5	6	7
HOW BOTHERED HAVE YOU BEEN DURING THE LAST WEEK DID YOU:							
	All of the Time	Most of the Time	Quite Often	Some of the Time	Once in a While	Hardly Any of the Time	None of Time
14. Shortness of breath	1	2	3	4	5	6	7
IN GENERAL, HOW OFTEN DURING THE LAST WEEK DID YOU:							
	All of the Time	Most of the Time	Quite Often	Some of the Time	Once in a While	Hardly Any of the Time	None of Time
15. Feel FRUSTRATED because you couldn't KEEP UP with others?	1	2	3	4	5	6	7
16. WAKE UP during the NIGHT because of your asthma?	1	2	3	4	5	6	7

17. Feel UNCOMFORTABLE because of your asthma?	1	2	3	4	5	6	7
18. Feel OUT OF BREATH because of your asthma?	1	2	3	4	5	6	7
19. Feel you COULDN'T KEEP UP WITH OTHERS because of your asthma?	1	2	3	4	5	6	7
20. Have TROUBLE SLEEPING at night because of your asthma?	1	2	3	4	5	6	7
21. Feel FRIGHTENED by an ASTHMA attack?	1	2	3	4	5	6	7
THINK ABOUT ALL THE ACTIVITIES THAT YOU DID IN THE PAST WEEK:							
	Extremely Bothered	Very Bothered	Quite Bothered	Somewhat Bothered	Bothered A bit	Hardly Bothered	Not Bothered
22. How much were you <u>bothered</u> by your asthma during these activities?	1	2	3	4	5	6	7
IN GENERAL, <u>HOW OFTEN</u> DURING THE LAST WEEK DID YOU:							
	All of the Time	Most of the time	Quite Often	Some of the Time	Once in a While	Hardly any of the Time	None of Time
23. Have difficulty taking a DEEP BREATH?	1	2	3	4	5	6	7

Asthma Quality of Life Questionnaire

Tick activities in which you are limited by asthma **during the last 2 week** from the following list. If you are limited in more than 5 activities because of asthma, choose 5 activities you are most bothered.

1	Bicycling	14	Shovelling snow
2	Clearing snow off your car	15	Singing
3	Dancing	16	Doing regular social activities
4	Doing home maintenance	17	Having sexual intercourse
5	Doing housework	18	Talking
6	Gardening	19	Running upstairs or uphill
7	Hurrying	20	Vacuuming
8	Jogging, excercising, or running	21	Visiting friends or relatives
9	Laughing	22	Going for a walk
10	Mopping or scrubbing the floor	23	Walking upstairs or uphill
11	Mowing the lawn	24	Woodwork or carpentry
12	Playing with pets	25	Carrying out your activities at work
13	Playing sports		

Fill in the blanks of the following 5 questions with the activities you chose above, then answer the questions by ticking the answer that suits you best:

	Totally limited, couln't do activity at all	Extremely limited	Very limited	Moderate limitation	Some limitation	A little limitation	Not at all limited
1. Please indicate how much you have been limited by your asthma in _____ ?	1	2	3	4	5	6	7
1. Please indicate how much you have been limited by your asthma in _____ ?	1	2	3	4	5	6	7
3. Please indicate how much you have been limited by your asthma in_____?	1	2	3	4	5	6	7
4. Please indicate how much you have been limited by your asthma in_____?	1	2	3	4	5	6	7
5. Please indicate how much you have been limited by your asthma in_____?	1	2	3	4	5	6	7

Tick the answer that best suits you for the following questions.

6. How much discomfort or distress have you felt over the last 2 weeks as a result of chest tightness?	A very great deal	A great deal	A good deal	A moderate amount	Some	Very little	No
	1	2	3	4	5	6	7

	All of the Time	Most of the Time	A good bit of the time	Some of the time	A little of the time	Hardly Any of the time	None of the time
7. In general, how often during the last 2 weeks have you felt concerned about having asthma?	1	2	3	4	5	6	7
8. How often during the past 2 weeks did you feel short of breath as a result of your asthma?	1	2	3	4	5	6	7
9. How often during the past 2 weeks did you experience asthma symptoms as a result of being exposed to cigarette smoke?	1	2	3	4	5	6	7
10. How often during the past 2 weeks did you experience a wheeze in your chest?	1	2	3	4	5	6	7
11. How often during the past 2 weeks did you feel you had to avoid a situation or environment because of cigarette smoke?	1	2	3	4	5	6	7

	A very great deal	A great deal	A good deal	A moderate amount	Some	Very little	No
12. How much discomfort or distress have you felt over the past two weeks as a result of coughing?	1	2	3	4	5	6	7

	All of the Time	Most of the Time	A good bit of the time	Some of the time	A little of the time	Hardly Any of the time	None of the time
13. How often during the past 2 weeks did you feel frustrated as a result of your asthma?	1	2	3	4	5	6	7
14. How often during the past 2 weeks did you experience a feeling of chest heaviness?	1	2	3	4	5	6	7
15. How often during the past 2 weeks did you feel concerned about the need to take medication for your asthma?	1	2	3	4	5	6	7
16. How often during the past 2 weeks did you feel the need to clear your throat?	1	2	3	4	5	6	7
17. How often during the past 2 weeks did you experience asthma symptoms as a result of being exposed to dust?	1	2	3	4	5	6	7
	All of the Time	Most of the Time	A good bit of the time	Some of the time	A little of the time	Hardly Any of the time	None of the time
18. How often during the past 2 weeks did you experience difficulty breathing out as a result of your asthma?	1	2	3	4	5	6	7
19. How often during the past 2 weeks did you feel you had to avoid a situation or environment because of dust?	1	2	3	4	5	6	7
20. How often during the past 2 weeks did you wake up in the morning with asthma symptoms?	1	2	3	4	5	6	7
21. How often during the past 2 weeks did you feel afraid of not having your asthma medication available?	1	2	3	4	5	6	7

22. How often during the past 2 weeks were you bothered by heavy breathing?	1	2	3	4	5	6	7
23. How often during the past 2 weeks did you experience asthma symptoms as a result of the weather or air pollution outside?	1	2	3	4	5	6	7
24. How often during the past 2 weeks have you been woken at night by your asthma?	1	2	3	4	5	6	7
25. How often during the past 2 weeks have you had to avoid or limit going outside because of the weather or air pollution?	1	2	3	4	5	6	7
26. How often during the past 2 weeks did you experience asthma symptoms as a result of being exposed to strong smells or perfume?	1	2	3	4	5	6	7
27. How often during the past 2 weeks did you feel afraid of getting out of breath?	1	2	3	4	5	6	7
28. How often during the past 2 weeks did you feel you had to avoid a situation or environment because of strong smells or perfume?	1	2	3	4	5	6	7
29. How often during the past 2 weeks has your asthma interfered with getting a good night's sleep?	1	2	3	4	5	6	7
30. How often during the past 2 weeks have you had the feeling of fighting for air?	1	2	3	4	5	6	7

	Severely limited-most activities not done	Very limited	Moderately limited-several activities not done	Slightly limited	Very slightly limited-very few activities not done	Hardly limited at all	Not limited at all-have done all activities that I wanted to do
31. Think of the overall range of activities that you would have liked to have done during the past 2 weeks. How much has your range of activities been limited by your asthma?	1	2	3	4	5	6	7

	Totally limited, couln't do activity at all	Extremely limited	Very limited	Moderate limitation	Some limitation	A little limitation	Not at all limited
32. Overall, among all the activities that you have done during the past 2 weeks, how limited have you been by your asthma?	1	2	3	4	5	6	7

NEO-FFI Personality Test

	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
1. I am not a worrier.	0	1	2	3	4
2. I often feel inferior to others.	0	1	2	3	4
3. When I'm under a great deal of stress, sometimes I feel like I'm going to pieces.	0	1	2	3	4
4. I rarely feel lonely or blue.	0	1	2	3	4
5. I often feel tense and jittery.	0	1	2	3	4
6. Sometimes I feel completely worthless.	0	1	2	3	4
7. I rarely feel fearful or anxious.	0	1	2	3	4
8. I often get angry at the way people treat me.	0	1	2	3	4
9. Too often, when things go wrong, I get discouraged and feel like giving up.	0	1	2	3	4
10. I am seldom sad or depressed.	0	1	2	3	4
11. I often feel helpless and want someone else to solve my problems.	0	1	2	3	4
12. At times I have been so ashamed I just wanted to hide.	0	1	2	3	4