Investigation of Alexithymia and Related Psychological Factors in Relation to Body Mass Index and Obesity

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Declaration of Originality

I hereby confirm that to the best of my knowledge, the content of this thesis is my own work and all else is appropriately referenced. This thesis has not been submitted for any degree or other purposes. I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

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Abstract

Common obesity is thought to be the result of genetic variations influencing susceptibility to environmental circumstances related to food intake, mediated by appetite-regulating pathways, and also affected by emotional processing and other behavioural traits. Alexithymia is a psychological construct for emotional processing deficits, characterised by impaired identification and description of feelings, and externally-oriented thinking.

Here, I investigate the relationship between alexithymia, measured by the 20-item Toronto Alexithymia scale (TAS-20), and adiposity in two Northern Finland Birth Cohorts (NFBC1966 and NFBC1986) and in severely-obese adults seeking bariatric surgery in the UK (PMMO clinical trial). Analysis of depression as a possible contributing factor in the relationship between alexithymia and obesity was also conducted in the NFBC1966. Consistent associations between BMI and TAS-20 total scores were observed among adult and adolescent general populations and in severely-obese adults (pre- and post-surgery, assessed longitudinally). Males with clinically-relevant alexithymia status (TAS-20≥ 61) and history of depression diagnosis had higher BMI than males without, at age of 31 years in NFBC1966. In the severe obesity clinical trial cohort (PMMO), participants with history of clinical depression diagnosis had higher TAS-20 total scores and weight at baseline than those who had no clinical depression history. Depression and bariatric surgery type were also moderately associated with TAS-20 total scores after surgery.

A genome-wide association study was conducted to identify genetic variants influencing psychological measures (TAS-20 and HSCL-13 scores) in the general adult and adolescent populations. In the NFBC1966 dataset, one SNP, rs2242223 ($p= 8.10 \times 10^{-8}$) in the Parkinson's disease gene *SNCAIP* (synuclein alpha interacting protein) was associated with TAS-20 score. There were also significant sex and genotype interactions.

These findings raise intriguing questions regarding the direction of causal mechanisms between emotional processing and obesity. Design of treatment strategies for complex conditions, such as obesity, would benefit from enhanced understanding of underlying psychological/behavioural components in the general population and clinical patients.

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List of Abbreviations

AN	Anorexia nervosa
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
ASD	Autism spectrum disorder
BDNF	Brain-derived neurotrophic factor
BED	Binge eating disorder
BN	Bulimia nervosa
CEU	HapMap European-derived catalogue
CHR	Chromosome
CI	Confidence interval
CNV	Copy number variant
COMT	Catechol-O-methyltransferase
DIF	Difficulty identifying feelings
DDF	Difficulty describing feelings
DRD2/ANKK1 Taq1A	Dopamine receptor D2
ED	Eating disorder
EOT	Externally-oriented thinking
GBA	Gut and brain axis
GXS	Gene x sex interaction
GWAS	Genome-wide association study
HG19	Human genome 19 reference
HSCL-13	13-item Hopkins Depressive Symptom Checklist
5-HTTLPR	5-HT transporter-linked promoter region
5-HT1A	Serotonin receptor 5-hydroxytryptamine 1A

IBS	Identity-by-state
LD	Linkage Disequilibrium
MDS	Multidimensional scaling
MAF	Minor allele frequency
NFBC1966	Northern Finland Birth Cohort 1966
NFBC1986	Northern Finland Birth Cohort 1986
PGC	Psychiatric Genomics Consortium
PMMO	Personalised Medicine for Morbidly Obese
PYY	Peptide tyrosine tyrosine
QC	Quality control
RNY	Roux- en-Y
SNPs	Single nucleotide variations
SNCAIP	Synuclein alpha interacting protein
TAS-20	20-item Toronto Alexithymia Scale
T2DM	Type 2 diabetes
WHO	World Health Organization
WHR	Waist-hip ratio
%WL	Percentage of weight loss

List of Publications

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CHAPTER 1 INTRODUCTION

1.1 Overview

This thesis describes an observational study exploring the implications of alexithymia (a deficiency in understanding, processing, or describing emotions) for human obesity, in both population-based and clinical settings. The inspiration for the study came from my initial interest in studying the link between autism and obesity. From a background in genetics and human biology, I have ventured into largely within the domain of psychology, well somewhat beyond the area of my earlier research experience. Consequently, I have undertaken an extensive literature search and learning to identify the theoretical framework for this dissertation. The Williams's INTEGRATED model of emotional processing (2008) and Gross's emotional regulation theory (1998) serve as the explanatory framework (4, 5). The primary data for this thesis was from the well-known longitudinal population-based cohort (the Northern Finland Birth Cohorts 1966 and 1986) and a clinical obesity population from Imperial College NHS Weight Centre, selected NHS centres and general practitioner surgeries in the UK. The most widely used alexithymia measurement tool is a self-report questionnaire, the 20-item Toronto Alexithymia Scale (TAS-20) (6, 7) which has been cross-validated in different languages, cultures, and populations (clinical vs nonclinical) (8) and data obtained using this instrument is used throughout this work. This chapter introduces alexithymia and its association with obesity from an epidemiological perspective. It will also present the applicability of genetic studies to the overlap between obesity and emotion processing deficits.

1.1.1. Scope of the Thesis

To date, the relationship between alexithymia and body mass index (BMI) has not been specifically investigated in large-scale general population settings. As a first stage, we sought to address this knowledge gap by determining whether or not an association exists between

them. For this, a standard cross-sectional analysis was carried out in the Northern Finland Birth Cohorts 1966 and 1986 (NFBC1966 and NFBC1986). In addition, a longitudinal analysis was conducted in NFBC1966 to study changes in alexithymia and obesity over the 15-year period, when the participants are between the ages of 31 and 46 years. Since alexithymia may be co-morbid with depression (9), it is possible that the association of body mass index (BMI) with the total score and its subscale of TAS-20 might be contributed by depression. Thus, I also explored the relationship between alexithymia and depression, expanding this further to explain variance in obesity measures.

The relationship of genetic and environmental factors with alexithymia had been explored in three twin studies in different populations and the authors concluded that alexithymia is genetically influenced, with heritability estimates between 30-33% (10-12). At the time of writing, there were no published genome-wide association studies (GWAS) on TAS-20 score. Since I have access to individual-level phenotype and genotype data of the 31-year old adults in NFBC1966, I carried out genome-wide association study (GWAS) separately on TAS-20 and HSCL-13 scores (as continuous traits). These analyses are also included in larger scale GWAS on alexithymia.

To explore whether the population-based results on the relationships between alexithymia, depression and obesity were transferable to a clinical cohort with extreme obesity, I also investigated an adult severely-obesity clinical cohort. A TAS-20 score prediction model was constructed which may be relevant in a clinical setting for obesity/weight management.

Causal inference, for example by Mendelian Randomisation approaches, is outside the scope of this study, as is any direct interventional study.

1.1.2. Rationale for the study

This thesis describes investigation of a commonly-used measure of alexithymia and in relation to body mass index (BMI) and obesity. Alexithymia is described as a deficit in emotion processing, specifically difficulties in identifying, differentiating and communicating feelings - in distinguishing feelings from the bodily sensations of emotional arousal (13). Individuals with this attribute fail to attend to their own emotions (e.g. happiness/sadness) and inner states (e.g. heartbeat, hunger/fullness) (14-17).

Alexithymia is present in 50% of adults with autism spectrum disorder (ASD) which is high compared to the general population (where reported levels are approximately 10%) (18). Although alexithymia and ASD are overlapping conditions, they are independent constructs (18, 19): brain structure network analysis suggested that that the alexithymia construct is independent of autism (19). It is important to recognise alexithymia, since it may have a negative impact on the outcome of various disorders and their treatment. Most contemporary researchers consider alexithymia to be a relatively stable personality trait that may have wide-ranging (negative) effects on health and subjective well-being (20). However, some researchers criticise the concept of alexithymia being an independent entity and a personality trait. It has, for example, been argued that alexithymia may simply be a covariate of other health problems (21).

A variety of physical and mental disorders have been associated with alexithymia including obesity, depression and anxiety, anorexia and bulimia nervosa and binge-eating disorder (22-28). Here, we concern ourselves primarily with obesity, and with depression. Obesity is caused by consumption of more food than is required for energy balance and it has long been recognised as a major public health concern, due to complications and co-morbidities that greatly impair physical and mental health outcomes, as well as quality of life (29). There can

be a range of reasons for obesity, including genetics (30-38), environmental factors (39-41) and, critically, over-eating in response to emotions (42, 43). In the context of this thesis, alexithymia may be directly relevant to energy-emotion imbalance, either because an underlying impairment of interoception causes blunted responses to satiety signals, or, alternatively, because of maladaptive emotion regulation strategies (eg. Emotional eating) following impaired emotional recognition. Lifestyle and medical interventions have only been very modestly successful for long-term treatment of obesity. Roughly 60-70% of excess body weight loss can be accomplished through bariatric surgery, however, and the results are more durable than following dietary restriction. Although this is highly successful compared to other available therapies, not everyone achieves this weight loss and some patients are unable to reach even 50% of excess weight loss, and others initially lose weight well, but suffer regain (44). In this work, we explore the relationship between BMI in the general population, and alexithymia. We also investigate this relationship in a cohort of patients undergoing bariatric surgery, to see whether it is associated with either baseline, or post-surgery weight.

There is also a well-established relationship between alexithymia and depression, and we seek to elucidate their joint implications for BMI. There are inconsistencies in reports examining the relationship between severity of depression and alexithymia score (45) and the direction of causality between alexithymia and depression is still undetermined.

1.2 Empirical review

In this section, I review the empirical evidence on alexithymia and its association with obesity from an epidemiological perspective. Additionally, previous research on genetic studies of alexithymia is discussed and research gaps are highlighted.

1.2.1 Alexithymia

Alexithymia, although under-recognised, is not a particularly rare phenomenon. The prevalence of alexithymia reported in adult general populations is around 10% (46), although the reported prevalence of alexithymia varies from 7.3% to 23.5% in adolescents (47). Alexithymia has been reported as associated with variety of medical conditions, with particularly high alexithymia rates (40-77%) in autism spectrum disorder (ASD) (48), anorexia nervosa (AN) (49) and psychosomatic disorders (50). Most contemporary researchers consider alexithymia to be co-morbid with ASD (48, 51, 52) due to stable test–retest reliability over time in the ASD population (51).

A wide variety of assessment tools have been developed for alexithymia (Table 1.1). The most widely used is the TAS-20 questionnaire. Consistencies are observed between the TAS-20 score and other alexithymia assessment methods for example observer-rated (Observer Alexithymia Scale) (53) and a skills-based approach (Emotion Recognition Task) (54) among eating disorders (ED) patients.

Table 1.1. Alexithymia measurement tools adapted from Encyclopaedia of Mental Health (2015) (55).

Instrument and source	Item content/ conceptual coverage
Self-report	
Twenty-item Toronto Alexithymia Scale (TAS-20)	Difficulties identifying and differentiating feelings (DIF), difficulties describing feelings (DDF), an externally oriented cognitive style (EOT).
Bermond-Vorst Alexithymia Questionnaire (BVAQ)	Limited fantasy and imaginations; failures in identifying, analysing and verbalizing one's emotional states
Structured interview	
Toronto Structured Interview for Alexithymia (TSIA)	Difficulty identifying feelings, difficulty describing feelings to others, and reduced fantasy/ poor imagination.
Diagnostic Criteria for Psychosomatic Research Alexithymia (DCPR-A)	Difficulties verbalizing and communicating emotional states, reduced fantasy, external thinking.
Semi-structured interview	
Modified Beth Israel Hospital Psychosomatic Questionnaire (modified-BIQ)	Difficulties with the identification and verbal communication of feelings; poor imaginal activity, operator thinking
California Q-set Alexithymia Prototype (CAQ-AP)	Difficulties experiencing and expressing emotion; poor interpersonal relationship;
Observer Alexithymia Scale (OAS)	Lacking skill in interpersonal matters and relationships; insight, and self-understanding; without imagination or humour.
Projective test	
Rorschach Alexithymia Scale (RAS)	Low awareness of affective states, poor fantasy, concrete/simplistic thinking; conventionality, social conformity; limited adaptive resources, difficulty managing interpersonal relationships
Observer-rated scored (indirect measure)	
Levels of Emotional Awareness Scale (LEAS)	Differentiation, specificity, and blending of emotions

For the purposes of this thesis, TAS-20 score is the chosen assessment method, because this questionnaire has already been applied to two large-scale population cohorts, and data were, thus, available for around 10,000 subjects, providing excellent statistical power for our analyses. Alexithymia can be assessed both on a continuous and categorical basis: a total score of 61 and higher on the TAS-20 is used as a cut-off to identify subjects with alexithymia in population-based studies (6).

1.2.2 Epidemiological studies on alexithymia and obesity

There has been limited study of correlation of TAS-20 score with BMI, and none in unselected large-scale general population cohorts followed longitudinally over a long-time period, as presented in this thesis. There are, however, data on clinical populations. Alexithymia is among the psychological constructs that have been described as being more frequent in the obese and eating disorder (ED) populations, with a prevalence of 12.5% – 52.1% (56) and 14.4% - 83% (57, 58), respectively. ED includes anorexia nervosa (AN), bulimia nervosa (BN) and binge eating disorder (BED). One study reported that BMI did not correlate with alexithymia among anorexic females(28). This is discordant with reports from Espina *et al.* (2004) and Courty *et al.* 2015, who argue that starvation affects cognitive functioning in alexithymia, since they found that BMI was correlated with TAS-20 total scores in AN patients (24, 49). A meta-analysis from Westwood *et al.* (2017) (58) revealed significant BMI differences between clinical vs control groups that can only be seen in AN studies. Critical and systematic reviews, by Nowakowski *et al.* (2013) (57) and Westwood *et al.* (2017) (58), of ED studies using the self-reported TAS suggested DDF and DIF subscales as trans-diagnostic criteria for emotional dysregulation across the ED spectrum. For AN patients, the TAS-20 total scores and subscale (DDF, DIF and EOT) scores were significantly higher than in normal-weighted controls (58).

The present thesis concerns the relationship between alexithymia, BMI and obesity. It has been suggested that, in severely-obese patients, emotional processing difficulties such as alexithymia might reflect an underlying eating disorder, such as BED (59). According to Westwood *et al.* (2017) (58), there are limited alexithymia studies of individuals with a BED diagnosis, explaining the inconsistencies between alexithymia association studies among EDs in different populations (as summarised in Table 1.2). Zak-Golab *et al.* (2013) and de Zwaan *et al.* (1995) observed that alexithymia measures did not differ between obese Polish and Austrian subjects with and without binge eating disorder (60, 61). Previous small-scale case-control studies indicate that the prevalence of alexithymia in obese patients is higher than in healthy individuals without pathological eating disorders (22, 62). For this thesis, I have carried out cross-sectional and longitudinal analyses of the relationship between BMI and TAS-20 score in two large European birth cohorts. In addition, I have used clinical study population for severe obese patients to investigate alexithymia, and depressive symptoms in relation to baseline characteristics and weight-loss intervention outcomes.

Table 1.2 Alexithymia key publications in participants with obesity and eating disorders.

Authors (year)	**Elfhag *et al.* (2007)**	**Pinna *et al.* (2011)**	**Pinaquy *et al.* (2003)**	**de Zwaan *et al.* (1995)**	**Speranza *et al.* (2001)**	**Zak Golab *et al.* (2013)**
Sample size	N=396	N=586	N=169	N=182	N=522	N=100
Sample characteristics	Obese vs control	Obese vs control	Obese: *BED vs no BED	Obese: *BED vs no BED	*EDs (AN,BN) vs control	Obese: $BED vs no BED
Population	Swedish	Italian	French	Austrian	French	Polish
Alexithymia measure	TAS-20	TAS-20	TAS-20	TAS-20	TAS-20	TAS-26
Alexithymia percentage	Obese (17%), HC (2%)	Obese (12.9%), HC (6.9%),	Obese (52%)	-	-	-
TAS-20 total scores and subscales	DIF> obese women; EOT>obese men	BED>no BED	BED>no BED	no group differences	DIF, DDF: all EDs > HC*; DIF:BN>HC;DDF:AN-R>HC	No group differences

*Diagnosis by DSM-IV criteria $Binge Eating Scale (BES). BED = Binge Eating Disorder AN-R = Anorexia Nervosa – Restricting Type; AN-B/P = Anorexia Nervosa – Bingeing/Purging Type; BN = Bulimia Nervosa

1.2.3 Genetic studies of alexithymia

Genetic association studies stemmed from the discovery of common genetic polymorphisms that acted as markers for rare variants (mutations) underlying monogenic forms of Mendelian diseases in family-based analyses of monogenic disorders such as sickle-cell anaemia (63), beta-thalassemia (64), Huntington's disease (65), and others. These fell out of favour in Mendelian conditions as the underlying mutations were discovered and genotyped directly. However, the association approach remains important where no such major mutation is present, but risk of disease results from a combination of more subtle genetic effects in interaction with environmental exposures. Recently, genome-wide association studies (GWAS) have been extensively used to dissect the genetic architecture of complex diseases, including autism, obesity and eating disorders (66) and although the results have generally not been useful as clinical predictors in individuals, they have been very helpful in elucidating pathophysiology. Despite the fact that GWAS methodology has provided valuable insights into disease biology, with more than 2,000 common disease-associated variants identified, there remains a problem of 'missing heritability' that is apparently unexplained by common SNPs (67). A partial explanation for this is hetogeneity – meaning that some rare forms of common diseases are caused by highly penetrant rare variants (68). Recently, there is accumulating empirical evidence suggesting that low-frequency and rare variants are associated with some incidences of complex diseases (69).

Before conducting genetic studies, it is important to establish that the trait of interest has a significant genetic component. Heritability studies have estimated the contribution of genes to the variation in BMI and waist circumference between to lie between 55-85% for BMI and waist circumference (10-12, 70, 71), respectively and between 30-55% for alexithymia (10-12, 70, 71). There is some direct evidence that these traits can be related; some genomic structural

mutations are known to cause both obesity and ASD (72), thus it is important to characterise the interplay between emotional-processing traits and obesity or eating disorders.

Despite the evidence of heritability, the genetic aetiology of alexithymia has not been well-studied and part of the work described in this thesis includes a GWAS of alexithymia, which is aimed at identifying genes or genomic regions that contribute to the trait.

1.2.4 Genome-wide association studies (GWAS)

GWAS was never intended to directly identify causative mutations causing disease, but to use common genetic variants as markers to importantly highlight genes and regions for further study and has been very successful in contributing to our understanding of disease pathophysiology. To date, GWAS have been highly successful in providing new biological insights on obesity and type 2 diabetes mellitus (T2DM): for example variants around the fat mass and obesity-associated (FTO) gene were associated with adiposity and related traits in multiple populations: the gene is highly expressed in the arcuate nucleus of the hypothalamus and appears to be important for the control of satiety and appetite (73, 74). Recent publications pointed to a topologically-associated domain (TAD) identified as a wider region, including *FTO*, affecting BMI (75). The TAD area encompasses the *IRXB* cluster (*IRX3*, *IRX5*, *IRX6*), *FTO* and *RPGRIP1L* genes. Novel techniques exploring chromatin behaviour are now being used to extend our understanding of the mechanistic underpinning of GWAS hits.

Similarly, common genetic variants identified by GWAS for T2DM, which were related to β-cell function, confirmed the importance of these pathways in diabetes aetiology (76-79). More recently, common variants have been used to investigate the interplay between obesity, fat distribution patterns and metabolic/cardiovascular disease (80).

The results from these types of study can also help ascertain the shared aetiology or pathways between psychological factors and physical disorders that could lead to better, or novel treatment options.

At the time of writing, there is no published GWAS for TAS-20 scores either in clinical cohort or in a general population. Chapter 5 of this thesis, therefore, describes a GWAS of TAS-20 score, carried out in an unselected Northern European general population. These analyses contribute to a larger alexithymia GWAS consortia work.

1.2.5 Candidate based-gene studies

Despite the fact that three twin studies in different populations indicated that alexithymia is genetically influenced, with heritability estimates between 30-33% (10-12), genetic association studies of the alexithymia construct have been limited. To date there are no published GWAS, only candidate-gene association studies. As shown in Table 1.3, five SNPs from different genes/pathways were associated with alexithymia measures in previous candidate-based gene studies;

1) Catechol-O-methyltransferase*; COMT* Val108/158Met (rs4680) (81)
2) Brain-derived neurotrophic factor*; BDNF* Val66Met (rs6265) (82)
3) 5-HT transporter-linked promoter region*; 5-HTTLPR* (rs25531) (83)
4) Dopamine receptor D2*; DRD2/ANKK1 Taq1A* (rs1800497) (84)
5) Serotonin receptor 5-hydroxytryptamine 1A; *5-HT1A* (rs6295) (85)

There has also been an attempt to explore the contribution of genetic variants that might directly affect gene function: Mezzavilla *et al.* (2015) conducted a genotyping analysis using an Illumina exome chip (note that this is not a GWAS chip) to investigate alexithymia in the

North-Eastern Italy population (n=533). They used a case-control design with clinical cut-off of TAS-20 > 60 for alexithymia case and control for TAS-20 ≤ 60. Possible candidate genes such as ATP-binding cassette 4 (*ABCB4)* previously associated with major depression, bipolar disorder and schizophrenia in the Korean population were identified significantly associated with alexithymia.

Table 1.3 Candidate gene –based studies on alexithymia across Western and Asian general populations.

Authors (year)	Ham *et al.* (2005)	Walter *et al*. (2011)	Kano *et al.* (2012)	Wahlstrom *et al.* (2012)	Gong *et al.* (2014)
Candidate gene(s)	*COMT* and 5-*HTTLPR*	*BDNF* and *DRD2/ANKK1*	*5-HTTLPR*	*DRD2/ANKK1*	*5-HT1A*
Population	Korean	German	Japanese	European American	Chinese
Sample size (N)	109	664	304	120	504
Alexithymia scale	TAS-20	TAS-20	TAS-20	TAS-20	TAS-20
Results	*COMT* Val/Val carrier has higher total TAS-20 score compared to individuals with Met/Met or Met/Val genotypes. No group difference between TAS-20 and 5-*HTTLPR* genotype.	Total TAS-20 score and DIF subscale were associated with carriers of at least one *BDNF* 66Met and one *DRD2/ANKK1* A1 allele.	Total TAS-20 and DIF subscale were associated with L/L than L/S or S/S genotypes.	Total TAS-20 was associated with A1/A1 than A1/A2 or A2/A2 genotypes.	Total TAS-20 was associated with CG/GG than CC genotypes.

1.3 Theoretical framework

This section discusses the theoretical models for this thesis. In the first part, the key concepts of emotional processing and regulations are defined and their roles in alexithymia, depression and obesity are discussed. In the second part, theoretical reflections on how biological mechanisms play a role in the association between obesity and brain health are summarized – and specifically how alexithymia might be relevant to weight gain or obesity. Furthermore, previous research is discussed and research gaps addressed in this thesis are pointed out.

1.3.1 Emotions

Emotions are distinct patterns of neural activity that are generated as the individual continuously evaluates objects, events and situations with respect to their relevance for his/her needs, goals, values, and general well-being (86). Emotion is often defined as a complex state of physical and psychological changes that are mediated by neural and hormonal systems (87). Reeve (2015) suggests there are four components of emotions which are interrelated: 1) subjective experience or feelings (positive or negative), 2) bodily arousal, 3) memory activation by specific information/emotional stimuli, and 4) social expression (88). The generation of emotion feelings requires a neural remapping of different features of the body state in the CNS, resulting from cognitive "appraisal" where the anterior insular cortex plays a key integrative role (89). Clearly, this cannot happen without interoception (sense of the internal state of the body) and it is postulated that defects of interoception may underlie alexithymia (14-17).

1.3.2 Emotion processing

Emotional processing is one's ability to process fear, anxiety, stress and other extreme events and move past them over time. Emotional processing also refers to a gradual reduction of emotional responding. Jack Rachman (1980) first introduced emotional processing term as "*a*

process whereby emotional disturbances are absorbed, and decline to the extent that other experiences and behaviour can proceed without disruption" (90).

In healthy development, individuals are aware and able to identify and describe their emotions and this **emotional recognition ability** is important in that it helps to understand them and link them to events.

Having recognised bodily sensations indicating an emotional state, a person must then interpret and respond to it. A structural model to explain theories of emotions in relation to cognitive, relational (or environment) and motivation aspects was developed by Richard Lazarus in the early 1990s what is known as "the appraisal theory" or "Lazarus's theories of emotions" (91, 92). The cognitive appraisal theory of emotion by Lazarus & Folkman (1984) argues that thoughts must occur first before the experience of emotion. Emotional appraisal refers to processes by which individuals' cognitions about events predict their emotional reactions to those events. Reappraisal refers to changing the way that one thinks about events and their relationship to the self, which may then alter emotional reactions.

Successful emotional processing is indicated when distressed emotional reactions in individuals are converted or changed to non-distressed reactions. The emotional processing theory, proposed by Foa and Kozak (1986), is based on the concept that fear is activated through associative networks that include information about the feared stimulus, escape or avoidance responses to the feared stimulus, and the meaning of the fear (e.g., threat or danger) (93, 94). Maladaptive or pathological fear occurs when it is intense to a degree that it gets in the way of functioning, or when it persists even when there are no clear indications of danger. The theory holds that chronic avoidance (e.g., escape behaviour, avoidance, dissociation) often leaves these maladaptive schemas in place, as people do not remain in a situation long enough for new learning to occur.

1.3.2.1 William's INTEGRATED emotional processing model

In Williams's INTEGRATED? model of emotional processing, the core principle is motivation to "minimise danger and maximize reward" which underlies the conceptual framework of emotion, thinking and self-regulation in all aspects of brain, body activity and behaviour (5). This model suggests there are individual variations in emotional processing that may be contributed to by age, genetic variations and brain plasticity as it adapts over time. Both genetic variants and age also play a role in susceptibility to mental disorders and the associated disruptions in brain organisation (5). The INTEGRATE model suggests that neuropeptides and hormones such as oxytocin, and neuropeptide Y (*NPY*) contribute to the processes of self-regulation as they have been found to interact in the regulation of fear-related anxiety (95, 96). Much attention also has been given to the role of genetic variants known to modulate brain chemicals such as faster acting neurotransmitters (*BDNF*: brain-derived neurotrophic factors, serotonin: *5-HT*, dopamine and noradrenaline) in affecting functions of emotional processes (97-99).

Emotion in the INTEGRATE model is defined as "*adaptive action tendencies that are mobilized by signals of potential danger or reward. They involve a "feedforward" mode of brain and body activity that is triggered automatically and without the need for conscious awareness of the triggering signal*"(5). Feeling was defined as the awareness of brain-body changes such as heart rate pounding, muscle tension, etc. What makes emotional experience in humans is the effect on cognitive which relies on conscious awareness (selective attention). Self-regulation is where the memory of emotional experiences will reinforce behavioural responses motivated by minimisation of danger and maximisation of reward (adaptive goals) over time.

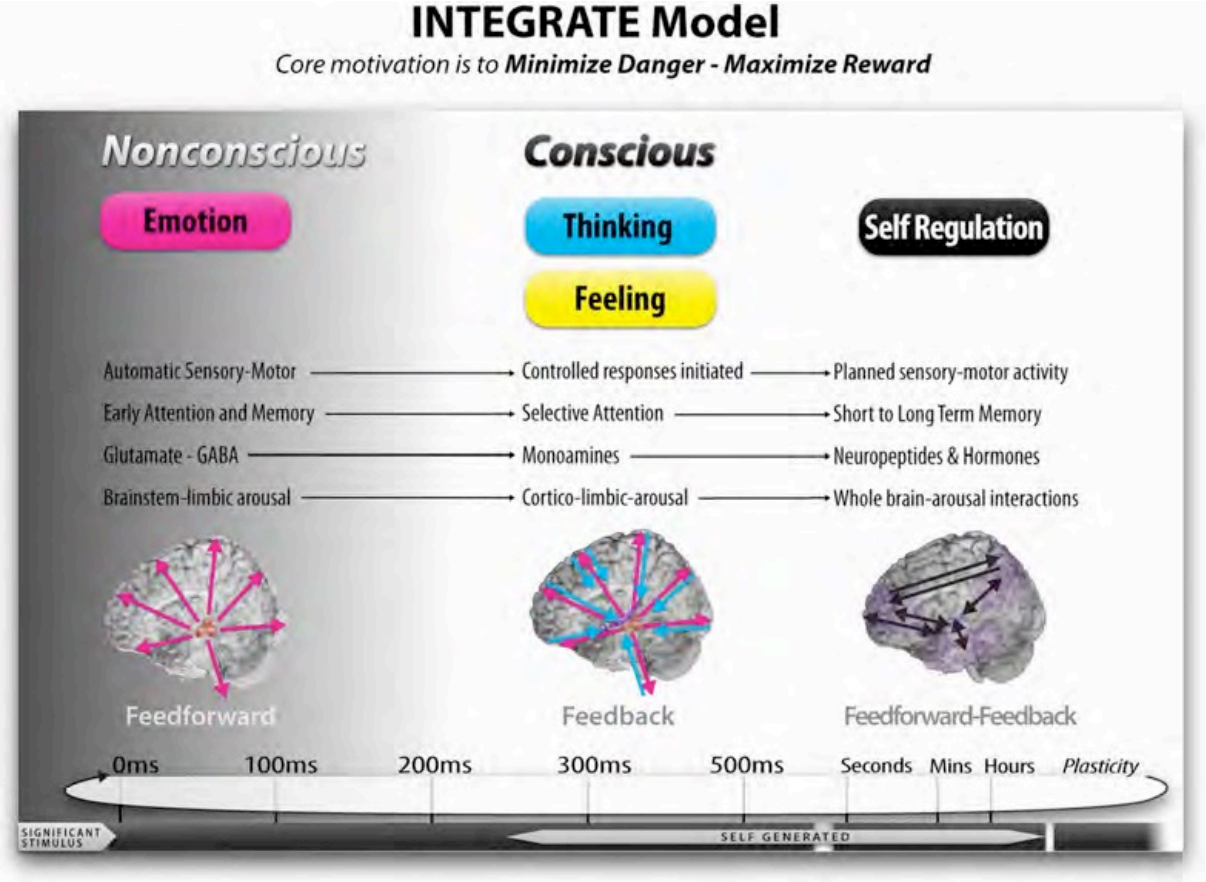


Figure 1.1 The INTEGRATE model of emotional processing by Williams *et al.* (2008) (5). Copyright permission granted from the publisher.

1.3.3 Emotional regulation

Emotional regulation is the ability to respond to the on-going demands of experience with the range of emotions which is socially tolerable and sufficiently flexible to permit spontaneous reactions as well as the ability to delay spontaneous reactions as needed. In another words, the overall emotional processes and skill sets used to change the emotional experience and response can be defined as emotion regulation (100). Cole *et al.* (1994) define emotional regulation as the ability to experience and differentiate a full range of emotions as well as to accept and value emotional responses (101). The ways in which individuals actively, and in a goal-oriented way, regulate their emotions refer to emotional regulation strategies (102). Emotional regulation strategies can be broadly divided into two categories, which are adaptive

and maladaptive. The most widely studied emotional regulations strategies are (1) cognitive-reappraisal: changing the way one thinks prior to the activation of emotional response systems to reduce its emotional impact; (2) suppression: inhibition of on-going emotionally expressive behaviour to decrease the emotional experience (103). Cognitive-reappraisal is an adaptive emotional regulation strategy which employs following strategy; (i) selecting the situation (e.g., approaching or avoiding people or situations according to their anticipated emotional impact), (ii) changing the situation (e.g., transforming the environment to alter the emotional impact), (iii) engaging attentional strategies (e.g., focusing attention towards or away from situational circumstances depending upon their emotional potency), and (iv) cognitive change (e.g., reinterpreting the situation to alter its emotional significance). Suppression is a response-focused strategy and it is an example of maladaptive emotional regulation strategy which has been associated with the higher prevalence of psychopathological symptoms (e.g. depression, anxiety, eating disorders, substance abuse) (2, 104).

1.3.3.1 Gross's emotional regulation model

There are four key steps in Gross's model of emotional regulation process;

(i) Emotional-relevant situation

(ii) Attention-directed to emotional situation

(iii) Appraisal (evaluation and interpretation) of situation

(iv) Emotional response to situation with changes in behavioural, experiential, and physiological response systems.

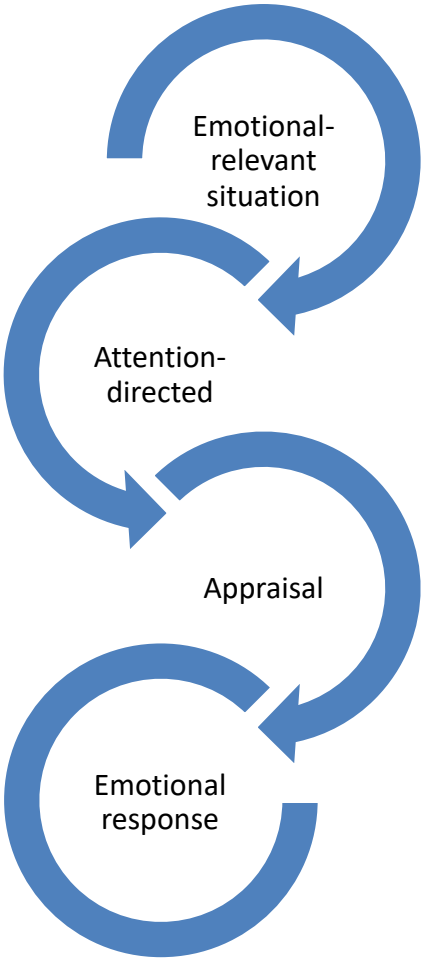


Figure 1.2 Cognitive emotional regulation is a complex process that involved the initiation, the inhibition, or modulation of (1) internal emotional states (the subjective experience of emotion);(2) emotion-related cognitions (e.g., reaction to thoughts about a situation); (3) emotion-related physiological processes (e.g., bodily arousal, immune response etc.); (4) emotion-related behaviours (e.g., facial expressions, verbal responses, etc.) [own illustration adaptation from Compare *et al.* 2014 (2)].

1.3.4 Alexithymia

Sifneos coined the term "**alexithymia**" from ancient Greek words; *a* = lack, *lexis* = word, *thymos* = emotions to describe a state of inability to understand, process, or describe emotions. Nemiah, Freyberger, and Sifneos (1976) conceptualised the alexithymia construct as deficits in the cognitive and affective processing of emotions based on the cognitive and affective domains of psychosomatic patients (13). Psychosomatic diseases are physical diseases that have a strong mental health, or stress-related component (e.g. eczema, psoriasis, high blood pressure, ulcers and heart disease) (105).

Individuals with alexithymia struggle to recognise or perceive non-emotional internal states from the body (termed "interoception") such as heartbeat, heat, nausea and hunger, and may find it difficult to identify cues between non-affective internal states and emotional experiences. Interoception refers to mapping process of the body's internal states by which the nervous system senses, interprets and integrates across conscious and unconscious level (106). Interoception plays a fundamental process for emotion processing, and alexithymia is suggested to be a marker of atypical interoception and the relationship between alexithymia and atypical interoception may hold important implications for understanding psychopathology (16, 107). Brewer *et al.* (2017) suggested that alexithymia may be a product of general interoceptive impairment rather than affective interoception impairment (16) and, it is possible that alexithymia may constitute an underlying factor that characterizes a number of distinct developmental, psychiatric and neurological conditions.

In a book entitled "Disorders of Affect Regulation: Alexithymia in Medical and Psychiatric Illness" by Graeme J. Taylor, R. Michael Bagby, James D. A. Parker (1999) (50), the authors

proposed the 26-item Toronto Alexithymia Scale (TAS) in which the alexithymia domains originally had four features:

"i) Difficulty identifying feelings and distinguishing between feelings and the bodily sensations of emotional arousal

ii) Difficulty describing feeling to others people

iii) Constricted imaginal processes, as evidenced by a paucity of fantasies

iv) A stimulus-bound, externally oriented cognitive style"

The shorter, TAS-20 questionnaire derived from this has three subscales which are difficulty identifying feelings (DIF), difficulty describing feelings to others (DDF) and an externally-oriented style of thinking (EOT) or *la pensée opératoire* (operatory thinking) (108). Controversies have been surrounding the TAS-20 scale and the alexithymia construct as some researchers suggest that it may not measure as it was originally conceptualised (109). A recent study by Di Monte *et al.* (2020) compared results using the TAS-20 and the Toronto Structured Interview for Alexithymia (TSIA) to determine levels of alexithymia between the two instruments in a group of people with obesity who were seeking surgical treatment (110). The authors found that the TSIA seems to show a greater level of alexithymia compared with the TAS-20 scale and they suggested using a multimethod assessment to evaluate alexithymia in a clinical setting.

There has been an extensive debate about whether the alexithymia construct represents a personality trait, whether it results from childhood experiences (111, 112) or is a covariate of other health problems (113). In 1977, Freyberger introduced the primary (personality trait-dependant) or secondary (state-dependant) concept of alexithymia (114). According to Lumley *et al.* (2007) it is likely that alexithymia includes both state and trait components, due to the complex manifestation of a person's cognitive and affective processing capacity (59). This has

implications for the stability of alexithymia measures – if it is a trait, one would expect any alexithymia score to be stable in a given individual – whereas if it is a state, one might expect it to vary dependent on circumstances. The data produced in Chapter 3 of this thesis shed some light on the long-term stability of the trait.

In addition to the importance of alexithymia for physical and mental health, recent research suggests that difficulties identifying and describing feelings (DIF and DDF subscales of the 20-item Toronto Alexithymia Scale; TAS-20) are related to symptoms of depression and anxiety (115-117).

1.3.5 Depression

Depression, otherwise known as major depressive disorder or clinical depression, is a common mental disorder that presents with low mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration (118). It is a mood disorder characterised by prolonged feelings of sadness and loss of interest in daily activities:if depressive symptoms persist for a period of at least two weeks, it is considered a depressive episode. Depression is known to be polygenic and multifactorial (119), and there is good evidence that it is related to alexithymia, although the direction of causation is unknown.

Emotion processing is associated with serotonin (120, 121) and dopamine (122-124) pathways. Emotional processing and self-regulation deficits are often associated with the difficulty identifying feelings (DIF) subscale of the TAS-20 (125-127). Moreover, individuals with depressive symptoms also often display high DIF and DDF subscale scores (115, 128-130). Emotional processing deficits and depression share substantial phenotypic variances: as such, individuals with high alexithymia scores (TAS-20) often have depressive symptoms (130-132). It can be suggested that the emotion deficits observed in alexithymia may be related to maladaptive emotion regulation strategy (for e.g. suppression) when faced with stressful events

or negative emotions (133, 134). This may be individuals with alexithymia having difficulties in recognising their own emotions, so they tend to use response-focused rather than antecedent-response emotion regulation strategies (134). A previous study by Chen *et al.* (2011) also found that alexithymia is associated with a more frequent use of suppression and a less frequent use of reappraisal in a group of 1788 college students.

A moderate (0.65) genetic correlation has been observed between alexithymia and depression, which suggests some common genetic basis between the two psychological constructs in general populations (70). More specifically, there was a substantial genetic correlation between TAS-20 subscales (DIF and DDF subscale) and depression. In addition to our lack of understanding of the genetic basis of alexithymia, and despite extensive GWAS and family-based genetic studies, the great majority of genetic factors contributing to the heritability of depression in clinical and population studies remain unknown.

1.3.6 Obesity

Obesity is defined as abnormal or excessive fat accumulation that poses a risk to health. The rise of obesity prevalence represents a major threat to worldwide public health. A crude population measure of obesity is the body mass index (BMI): a person's weight (in kilograms) divided by the square of his or her height (in metres). A person with a BMI of 30 kg/m^2 or more is considered to have obesity. The fundamental cause of obesity is an energy imbalance between calories consumed and calories expended. The brain plays a key role in the controls of energy intake and expenditure and many genes that are implicated in single-gene forms of human obesity (for eg. *MC4R*, *POMC*, and *LEPR*) are expressed in the central nervous system (CNS) (135). Moreover, insulin and leptin receptors are expressed in homeostatic and motivation-reward brain regions, that relay signals via dopaminergic neurons to cortical, limbic, and striatal brain regions involved in motivational and behavioural responses to

rewarding food stimuli (42). Figure 1.3 shows an illustration of different neurotransmitters and their receptors, hormones and other appetite regulators (glucagon-like peptide 1, cholecystokinin, peptide tyrosine tyrosine (PYY)) that play a role in the brain reward system. Although accumulating evidence supports the critical role of the neuroendocrine system in weight homeostasis and the development of obesity (136), the contribution of obesity-related metabolic and hormonal changes to psychological traits remains uncertain.

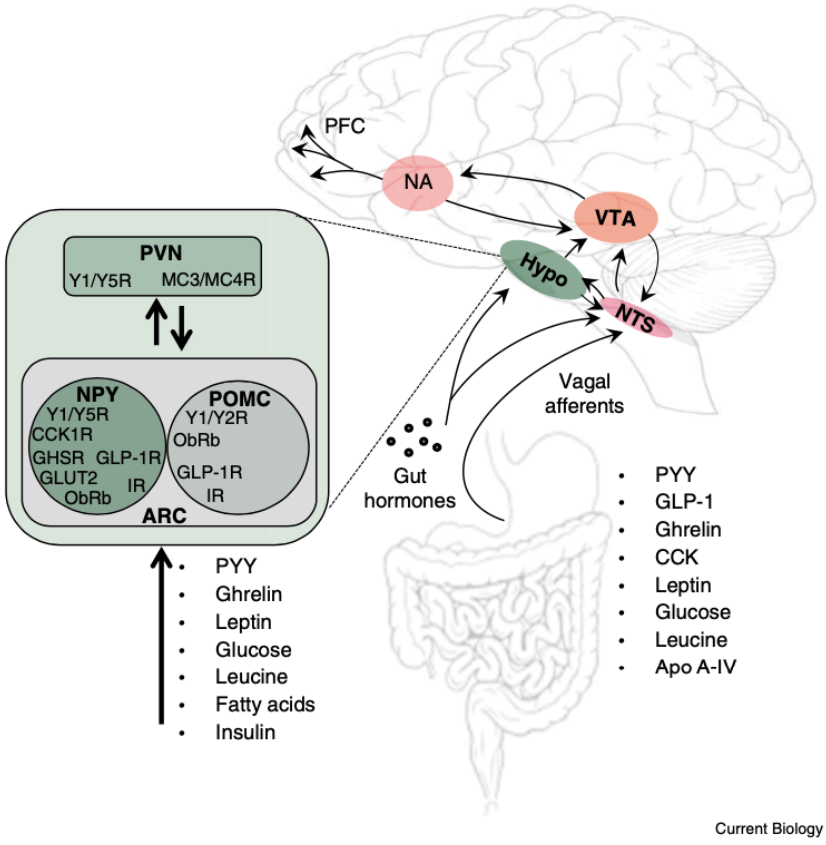


Figure 1.3 Brain reward signals in the central nervous system. Circuits in the hypothalamus (Hypo) and brainstem interact with higher centres to human feeding regulations. ARC; arcuate nucleus, PVN; paraventricular nucleus, NA; nucleus accumbens, NTS; nucleus of the solitary tract, PFC; prefrontal cortex, VTA; ventral tegmental area, GLUT2; glucose transporter 2, IR; insulin receptor. Image adapted from Chambers *et al.* 2013 (3).

1.3.7 Interplay between alexithymia, depression and obesity

Adults with obesity consistently exhibit increased activation to food-related stimuli in prefrontal and parahippocampal brain regions associated with cognitive function (137) and feelings. It has been suggested that, as a result of internal stimuli perception (interoception) deficits, alexithymia may be a cause of emotional eating among obese individuals (23, 138, 139). According to the Macht's five-way emotion and eating model (140), individuals experiencing depressed moods show preference for and consume palatable "comfort foods" in order to alleviate their negative feelings. In other words, negative emotions impair cognitive eating controls and negative emotions elicit eating to regulate emotions. In addition, there is evidence that shows palatable foods (which includes calory-rich foods) can provide some relief from negative emotions and internal states (141). Prolonged high-fat diets have been reported to lead to negative emotional states, increased stress sensitivity, and altered basal corticosterone levels (142). Thus, chronic consumption of "comfort foods" ultimately can lead to obesity which, in turn, may promote depressive symptoms in individuals.

According to Newson and Flint (Obesity Working Group 2011), specific psychological disorders such as depression, anxiety and eating disorders hasten the onset of obesity (143). Individuals with these forms of illness or mental distress had difficulty in controlling food consumption which was used as a coping strategy to avoid negative emotions. The relationship between obesity and mental health is also complicated by the effects of medication. Since the introduction of anti-depressants in 1900, there has been an accumulation of evidence for weight gain as an adverse effect treatment with of tricyclics and monoamine oxidase inhibitors treatment (144). A high prevalence of psychological illnesses particularly mood disorders, anxiety, and low self-esteem have been found associated with obesity (145). Large

epidemiological studies in Western countries have revealed a positive association between obesity and depression (146-151).

There is also evidence that psychological factors might impact obesity differently in males than females. Investigations into sex differences in the relationship between obesity and psychiatric disorders have been focused on depression and eating disorders. In the longitudinal NFBC1966, more females who were obese at the age of 14 and 31 years had current depressive symptoms. In addition, male abdominal obesity was associated with a higher life-time risk of clinical depression and with experience of current depressive symptoms at time of survey (152).

Conversely, the 'jolly fat hypothesis' was first suggested nearly half of a century ago by Crisp and McGuiness (1976) who found obesity to be inversely related with levels of anxiety and depression amongst the middle-aged population (153). This was later confirmed by Palinkas *et al.* (1996) who discovered that overweight men had a lower prevalence of depression compared to normal-weighted men (BMI< 25 kg/m^2) (154). The theory of 'happy life' among increased BMI males and females was confirmed in longitudinal and representative studies of middle-aged to elderly Chinese populations (155-160). However there have been discrepancies in the 'jolly fat hypothesis' findings in other Asian countries (Japan, Korea, Taiwan and Iran) (161-167) due to differences of depressive symptom scales, study design and type of analysis performed. Thus the working theory between psychology and obesity is complex, with many different potential confounders including age, sex and socio-economic status playing a role (168).

As well as contributing to the development of obesity, psychological factors also clearly affect responses to therapeutic intervention. Disordered eating pathology contributes to poorer weight loss outcomes among patients with severeobesity that underwent weight-loss interventions e.g. bariatric surgery. Research has been on-going to find significant psychological predictor(s) for

successful weight-loss outcomes for obesity interventions and in this thesis, I consider alexithymia and depression also in the context of weight loss outcomes after bariatric surgery. Due to the complexity of both psychological factors, and of obesity aetiologies, questions about the causal relationship or direction between emotion and obesity remained unanswered.

1.3.8 Genetic factors implicated in the overlap between obesity and emotion processing

Although little is known about the genetics of alexithymia itself, there are well-established overlaps between some syndromic forms of obesity and autism (for eg. in Prader-Willi syndrome and in 16p11.2 deletion carriers). More than 100 specific genes are reported to be involved in BMI differences and obesity (169). GWASs have also identified single nucleotide polymorphism (SNP) clusters associated with extreme excess body weight phenotypes after bariatric surgery (44). Many of these genes appear to impact the brain systems (170) that control appetite and the reward systems, and variations in these genes influence eating behaviour (171). There are also individual differences in emotion regulation abilities and their relation to eating (172, 173) and these are reportedly determined by genetics (171), psychological factors and social environment (174).

A recent GWAS by Morris *et al*. (2019) highlighted genetic variation in synaptic cell adhesion molecule 2 (*CADM2*) as one potential link between psychological traits and obesity (175). *CADM2* is a gene that encodes a mediator of synaptic signalling that has previously been associated with a range of psychological traits and health-related behaviours such as personality (176), attention-deficit/hyperactivity disorder (177) , risk-taking behaviour (178), alcohol consumption (179), educational attainment (180), cannabis use (181), as well as the interaction between physical activity habits and obesity (182). SNPs in *CADM2* are also associated with BMI in some GWASs (169, 183). *CADM2* has been previously reported to be

a potent regulator of systemic energy homeostasis (184) and the *CADM2* genetic variant (rs13078960) was associated with increased *CADM2* expression in the human hypothalamus. The effect of reduced body weight was seen in *Cadm2*-knockout mice (185) and it was maintained when were crossed with the traditional obesity model, the *Leptin*-knockout mouse (184). This suggests that some genetic variants could be implicated in the interplay between emotional processing and obesity and provides further basis for the GWAS of TAS-20 score.

1.4 Conceptual model

Based on a review of theory and research results, a model of the relationship between alexithymia and obesity has been developed. Figure 1.4 illustrates how alexithymia and depression may be linked to obesity. The conceptual model for investigating alexithymia may overlap with other psychological factors, including depression. This model serves as the analytical framework of this dissertation.

Alexithymia is a multi-faceted construct encompassing difficulty identifying feelings (DIF), difficulty describing feelings (DDF) and externally-oriented thinking (EOT). Individuals with alexithymia are thought to have impairment in cognitive, physiological and behavioural mechanisms, according to the generally-accepted James Gross's models of emotion regulation (186, 187). Individuals vary in their abilities to identify and express their emotions (188), and approximately 10% of the adult European population have alexithymia (18). Alexithymia has been previously reported to be associated with depression (189, 190) although previous research does not consistently show depressed individuals scoring high for alexithymia (45). In addition, there are inconsistencies between studies examining the relationship between severity of depression and TAS-20 score (and its subscales; DDF, DIF and EOT, in particular) (45). It has been suggested that there is an overlap, or a shared construct of emotional

dysregulation between depression and alexithymia (191). Additional effort is needed to define the putative pathophysiologic mechanisms of alexithymia and depression in relation to obesity.

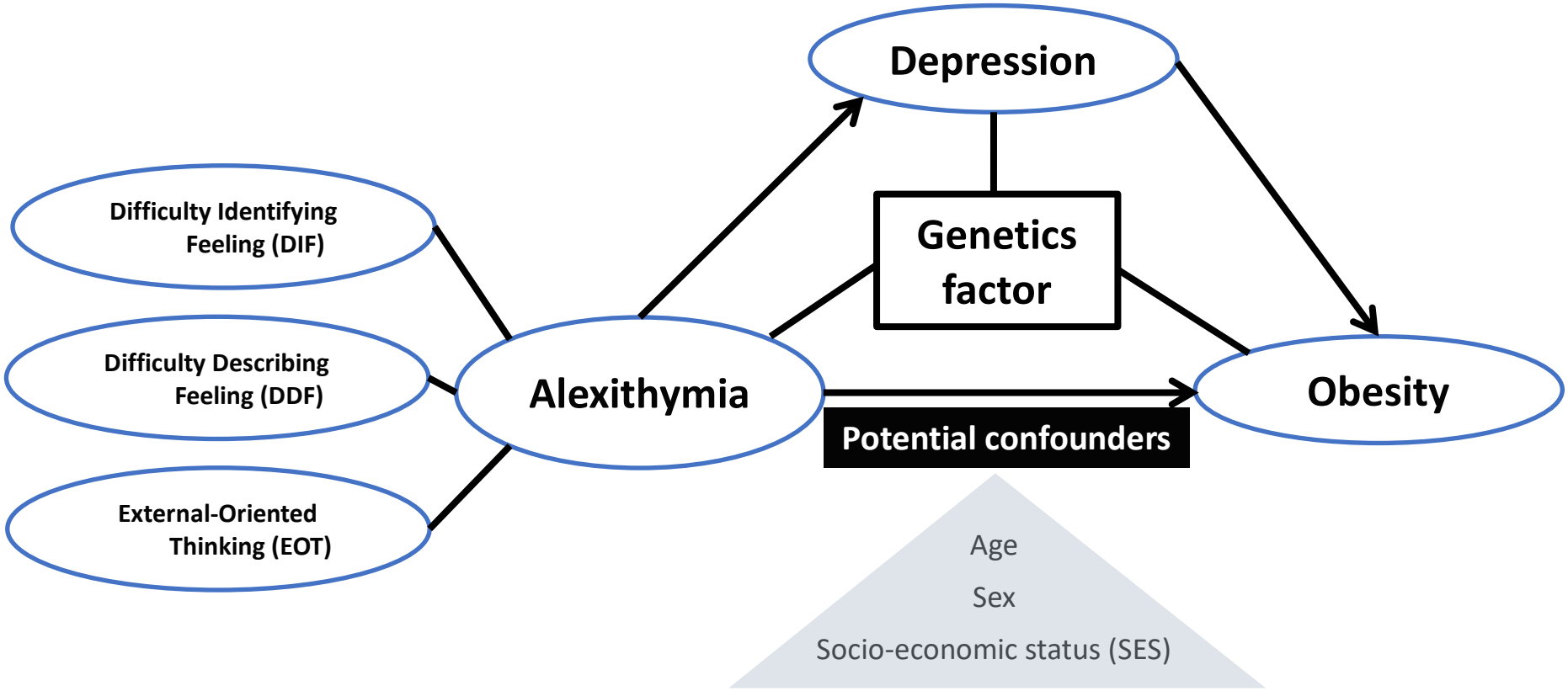


Figure 1.4 Conceptual models of the relationship between alexithymia, depression and obesity based on existing literature reviews. This diagram served as data analytical framework for this dissertation.

Obesity, anorexia nervosa, bulimia nervosa and binge-eating disorder, as well as a number of other physical and psychological disorders, have been associated with alexithymia (22, 25-27, 49, 61, 62, 192-194). In addition, depression is a common mental health condition which has considerable impact on both obesity and alexithymia (195). The direction of causality between depression and obesity is still undetermined. The relationship may not be simple: it is possible that a disturbance in the cognitive appraisal of events leads to a disturbance in emotions that may affect reporting of alexithymic tendencies (196).

Obesity is associated with insensitivity to satiety signals as reported in classic studies by Kaplan and Kaplan (1957), Schachter (1968), and Stunkard & Fox (1971) (197-199). The internal/external theory of obesity by Schachter (1968) and Canetti *et al*. (2002) predicts that normal eaters alter their food intake to regulate their emotion, while obese people do not (198, 200). Psychosomatic theories of obesity suggest that obese people overeat due to inability to perceive their physiological state, hunger, and satiety to the same magnitude as lean people and/or that overeating reduces emotional discomfort and anxiety (197).

Obesity has been shown to increase incidence of anxiety and mood disorders (201). In recent neuroimaging studies, adults with obesity may not exhibit typical satiety-related changes in brain activity following a meal (137, 202). In other words, insensitivity to satiety signals can be reflective of as interoceptive insensitivity as a path to overeating and obesity. Simmons and DeVille (2017) propose that "interoception not only turns the physiological state of having low blood sugar into the cognitive experience of "hunger", but also provides the human conceptual system with information about the body's somatic context, thereby influencing the construction of specific hunger-related emotional states" (203).

1.4.1 Energy intake and emotion homeostatic system

In 2007, Alonso-Alonso and Pascual-Leone proposed the right brain theory which focused on the importance role of prefrontal cortex in controlling cognition on reflective eating (170). Based on this theory, eating regulations include three levels of cognition, homeostasis and reward, and is controlled by either reflexive or reflective eating mode. This dual way of reflexive and reflective eating depends on reactions to food shortage and long-term health goals. These regulatory pathways are extensively inter-connected within the brain and its integrated functions control food intake according to internal and external factors. The authors argued that the frontal lobe of human brain had expanded through evolution. They suggest that genetic and environmental factors are most likely contribute to the right brain functioning. Prolonged activation of chronic psychosocial stress that are common in individuals with obesity could result in over-consumption of food as coping mechanism, thus facilitating adverse health outcomes including depression. Variability in terms of socio-demographics and inter-individual genetic variants could also play a role in the relationship between the right prefrontal dysregulation, stress response, and obesity.

The homeostatic theory of obesity assumes the disequilibrium or disturbance of stability for this system (or factors) that controls weight gains for most people. The Circle of Discontent theory by David Marks (2015) suggested that body dissatisfaction, negative affect and the consumption of energy-dense foods cause obesity (174). This model offers a view of how overweight and obesity are maintained over time for both individuals and societies. The theory, however, did not include genetic factors directly; rather it focuses on the homeostatic system for regulating energy intake and emotions.

1.4.2 Obesity as a neuro-behavioural disorder

There is growing evidence that obesity is a neuro-behavioural disorder. Stephen O'Rahilly and Sadaf Farooqi (2008) suggested that common obesity is the result of common genetic variants' function in the current environment leading to increased food intake, mediated by appetite-regulating pathways within the hypothalamus interacting with autonomic nervous system, and affected by psychological and behavioural measures (204). The discovery of monogenic forms of obesity (non-syndromic forms of obesity) has been accelerated by the advent of exome sequencing. Indeed, a new form of inherited obesity and type 2 diabetes, - homozygosity for a truncating mutation of the carboxypeptidase E (*CPE*) gene (35) was recently found in the same clinical obesity cohort examined in the last experimental chapter of this thesis. The patient with CPE mutation also had intellectual disability, which emphasises the neurobiological basis for appetite and satiety in the brain region. The discovery of an increased risk of obesity of around 30-fold resulting from an autism-associated 593 kb deletion at chromosome 16p11.2 by Walters *et al.* (2010) (72) initially in one of 34 children with intellectual disability and severe obesity, and then in 30 additional subjects from the European general population (including 12 from the Northern Finland Birth cohorts also examined in this thesis) lends further support to the neurobehavioural hypothesis.

1.4.3 Psychobiological relationships between alexithymia, depression and obesity

There is a growing body of literature demonstrating that dysfunction of peripheral and central metabolic mechanisms plays a role in the relationship, between obesity and brain health [see review by Stillman *et al.* 2017 (205)]. Neuropeptides and peripheral hormones have been reported to regulate emotion, food intake, and obesity [see review Singh 2014 (141)]. Neuropeptides such neuropeptide Y (NPY) and cholecystokinin (CCK) have a regulatory

role in the gut brain axis (GBA) and were alsoare associated with specific aspects of information processing and behaviour. In line with Williams's INTEGRATE model: when emotions are negative, they produce suppression of the immune system. Hormones such as ghrelin, and leptin have been implicated in interacting with the hypothalamus to regulate food intake, energy homeostasis, promote satiety, and hunger. Interestingly, both hormones have been implicated in cravings and mood disorder, and have also been associated with the reward pathway (206, 207). This suggests that both ghrelin and leptin are implicated in mood as well as food intake.

Impaired or atypical interoception (problems with perceiving one's internal bodily signals) is a candidate mechanism underlying alexithymia: researchers found that alexithymia was associated with lack of sensitivity to internal stimuli, e.g. heart beats (14-17). Murphy *et al.* (2017) (16, 208), proposed in a recent review that alexithymia is a marker of atypical interoception, due to a strong links between both traits, and may serve as a screening tool for alexithymia-related illnesses, including obesity. Alexithymia has a negative impact on the outcome of various disorders and on treatment outcomes and, thus, it is important to recognise this trait. In particular, patients with both obesity and alexithymia may respond poorly to psychological treatments, especially to cognitive-behavioural techniques.

Depression is a negative emotional state that affects health and well-being and has been linked to failures in interoceptive state changes (209). Mood is an emotional state that is experienced internally, as opposed to affect, which is the external expression of the mood (210). Previously, mood disorders were known as affective disorders. Mood disorders are mainly a group of psychological disorders that feature negative emotional state (affect) symptoms (210) such as depression and anxiety. Although there are similarities of emotional concept between depression and alexithymia, studies in the early 1990s (since the

development of the TAS-20) have shown that the two psychological states are distinct or separate constructs (189, 211). Over the past two decades, small-scale research and case studies began to emerge, linking alexithymia and depression in different medical illnesses. Several lines of evidence from clinical and general population studies suggest that alexithymia and depression are separable psychological domains, however, they are closely related and highly associated in a subset of subjects who were both alexithymic and depressed (116, 212). Recently, the TAS-20 scale has been suggested to measure negative affects rather than alexithymia itself in general and psychiatric populations, supporting the hypothesis that alexithymia is a state-dependant feature rather than a personality trait (213, 214).

1.4.4 Research Approach

I specify here the research themes, assumptions and key hypotheses which are tested throughout the thesis.

1) There may be a positive correlation between TAS-20 score and BMI (null hypothesis to be tested: there is no such association).
2) There may be an association between common genetic variation and TAS-20 score and depressive symptoms scores in the study populations (null hypothesis to be tested: there is no such association).
3) There may be an association between TAS-20 score and depression in an adult severely-obese clinical cohort (null hypothesis to be tested: there is no such association).
4) Alexithymia may influence weight trajectories and/or response to therapeutic interventions such as bariatric surgery (null hypothesis to be tested: these outcomes are unrelated to alexithymia).

1.4.5 Research strategy: Aims and Objectives

The overarching aim of this work is to explore the relationship between alexithymia, BMI and human obesity. To this, I had the following specific objectives:

1) To explore the relationships between alexithymia, depressive symptoms and obesity measures (BMI and WHR) in two Northern European unselected general population cohorts (the Northern Finland Birth Cohorts of 1986 and 1966).
2) To carry out a GWAS analysis for TAS-20 and HSCL-13 scores, in the same cohorts, using genotyping data from the Illumina Beadchip platform.
3) To study alexithymia and depressive symptoms among adults in clinical bariatric surgery cohort (Personalised Medicine in Morbid Obesity), using cross-sectional and longitudinal approaches.
4) To determine whether these two psychological constructs, alexithymia and depression, are significant predictors of bariatric surgery outcomes in the same sample.

CHAPTER 2 MATERIALS AND METHODS

2.1 Materials

The data from individuals born in Northern Finland (Oulu and Lapland provinces) in 1966 (expected date of birth estimated from mother's last menstrual period was for the year 1966) and 1985-1986 (expected date of birth estimated in mother's last menstrual period was for the period between July 1st 1985 and 30th June 1986) that form part of the Northern Finland Birth Cohort (NFBC) study were used for the analyses described in Chapters 3, Chapter 4, and Chapter 5 of this thesis.

In addition, data from a clinical trial of severely-obese adults, called Personalised Medicine for Morbid Obesity (PMMO), was analysed as described in Chapter 6 of this thesis.

The Northern Finland Birth Cohort 1966 (NFBC1966) and the Northern Finland Birth Cohort 1986 (NFBC1986) were initiated by Professor Paula Rantakallio (University of Oulu, Finland) to study the implications of the exposures during the prenatal period and early childhood development for subsequent morbidity and well-being(215-217). Extensive data from the early prenatal period until middle age were collected at different timepoints, enabling researchers to carry out various epidemiological and longitudinal studies(218-220). The ethics for each cohort was approved at multiple stages by the Ethical committees of University of Oulu, and the Northern Ostrobothnia Hospital District also approved the study at multiple stages(217). Informed consents were collected from all individuals whose data was used in this thesis. Only data from those people who gave consent at the respective timepoints was used in the analyses.

2.1.1 The Northern Finland Birth Cohort 1966

The NFBC1966 started in 1965 with the recruitment of pregnant women who had their calculated dates of delivery for the year 1966 based on mother's first day of the last menstrual period. These women have been followed up ever since, together with the recruitment (and

subsequent follow-ups) of their offspring (Figure 2.1). The latest data available for the offspring are at 46 years of age.

However, only the data at 31-year (postal questionnaire data received; N=8,767, clinical examination data received; N=6,033) and 46-year (postal questionnaire data received; N=6,868, clinical examination data received; N=5,861) timepoints were included in this thesis. Data on variables from 31-year (31y) and 46-year (46y) follow-ups were systematically explored and described in the sub-sections below.

2.1.1.1 Follow-up at 31 years

In 1997, postal questionnaires were sent to the NFBC1966 participants (at the 31-year timepoint), during which they were also invited to attend clinical examinations at clinical data collection centres set up at different parts of the country. The postal questionnaires included questions on socio-economic status (SES), anthropometrics (i.e. height, BMI), mental conditions (i.e. depression, anxiety), diet (i.e. food frequency, food preference), physical conditions (i.e. physical exercise, physical performance capacity), lifestyles (i.e. smoking, alcohol consumption, reproductive health), gynaecology, environment, and public health services used. The TAS-20 scale, together with opinions and experience questionnaire, was handed to the participants during clinical examination in 1997. The forms were filled in the clinics while waiting or returned by mail. The response rate for the postal questionnaires was 75% (N=8,767) and attendance in clinical examination 71% (N=6033) of the eligible population (those still living in original catchment area in Northern Finland and those who had move to capital city area, Helsinki and its surroundings).

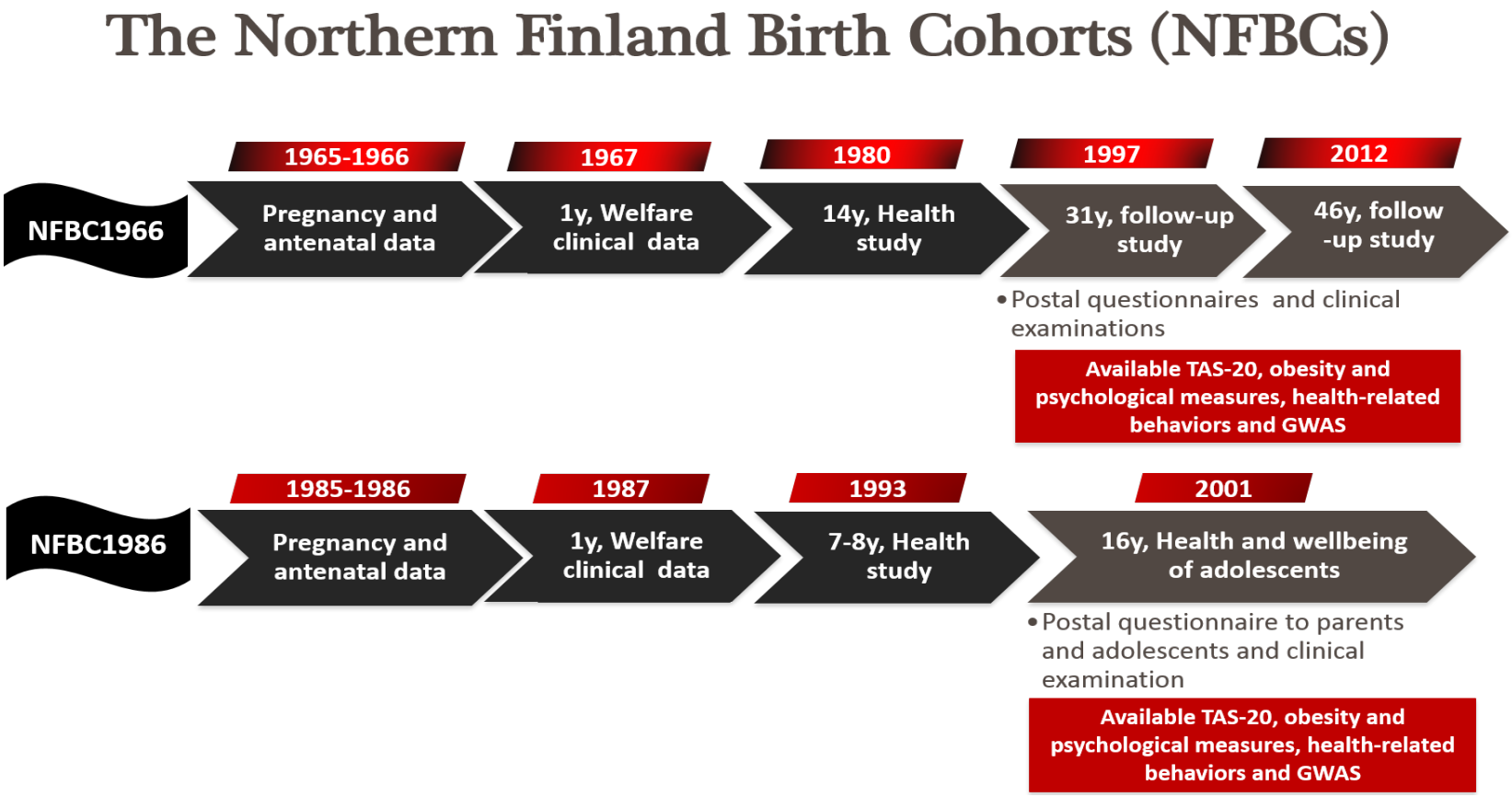


Figure 2.1 Timepoints when follow-up data collections were conducted in the NFBC1966. Questionnaires were collected from 24th and 28th gestational week onwards but data itself covers also early pregnancy from the 16th gestational week onwards and some pre-pregnancy information.

The clinical examinations (i.e. body measurements, including weight, height, waist and hip circumferences, blood pressure measurements, blood collection and tests, physical fitness tests among others) were conducted by trained research nurses in respective data collection points set up at community health centres in Northern Finland and in the south.

2.1.1.2 Follow-up at 46 years

In 2012, all individuals at 46 years of age that could be traced were followed-up by a further set of questionnaires on health, behaviour, work and social background. The TAS-20 scale, together with opinions and experience questionnaire, was handed to the participants during the clinical examination sessions. The forms were filled in the clinics while waiting or returned by mail. The questionnaire was completed by 68.5% (N=6868) for background information, opinions and experiences, lifestyle-economy, work and mental resources, dental health, physical activity and 15D measure of health-related quality of life questionnaire, online or on paper. The TAS-20 questionnaire was included in an "opinions and experiences" questionnaire. The clinical examination invitations were sent to all cohort members, of which 56.7% (N=5861) participated. Anthropometric data (height, weight, waist and hip circumference, bio impedance), physical activity, fitness, conditions of lung and heart, dermatology, allergic diseases, eye diseases, pain perception and tolerance, musculoskeletal health, cognitive testing, oral health, biological samples (blood, saliva, hair, urine and faecal) were also successfully collected by trained nurses at this time-point.

2.1.2 The Northern Finland Birth Cohort 1986

Data collection for the younger NFBC cohort (NFBC1986) started in 1984 (Figure 2.2). This cohort included all mothers (N=9,362) with their children born between the 1st July 1985 and the 30th June 1986 in Oulu and Lapland, Northern Finland. Background information questionnaires were handed out to all the mothers at the first antenatal visit, on average in

the 10th gestational week and returned by the 24th gestational week if still pregnant. The pregnancy data such as diseases and pregnancy complications, delivery and child's survival were collected either from antenatal clinics or from the questionnaires completed in consultation with the parent's local midwives.

The number of children born was 9479 (99.5% of all eligible in the region), including 9432 alive born children. There were 4,865 boys and 4,567 girls. At 1 year old, the offspring data concerning growth, health and development, were collected at the children's welfare clinics (N=1802, subsample). In addition, follow-up postal questionnaires related to the children's psychomotor development and behaviour at the age of 7, were sent to the parents (N=9,326). A total of 8,390 parents gave their consents to be included in the NFBC1986 project. In 1993, at 15-16 years of their children age, the parents were sent a further two sets of questionnaires regarding the children's behaviour at school and at home.

The children's behaviour was assessed using Rutter scale, RB2, as well as for learning difficulties (N=8,525). Finally, between April 2001 and February 2002, the questionnaire study for adolescents (16-17 years of age) and their parents was conducted. As part of the Northern Finland 1985-1986 Birth Cohort Welfare and Health Research Programme, a clinical examination of the adolescents was conducted between August 2001 and June 2002 in municipalities of Northern Finland (in data collection centres) and in the major cities elsewhere in Finland (all alive with known address were invited to attend clinical examinations at the age of 16 years).

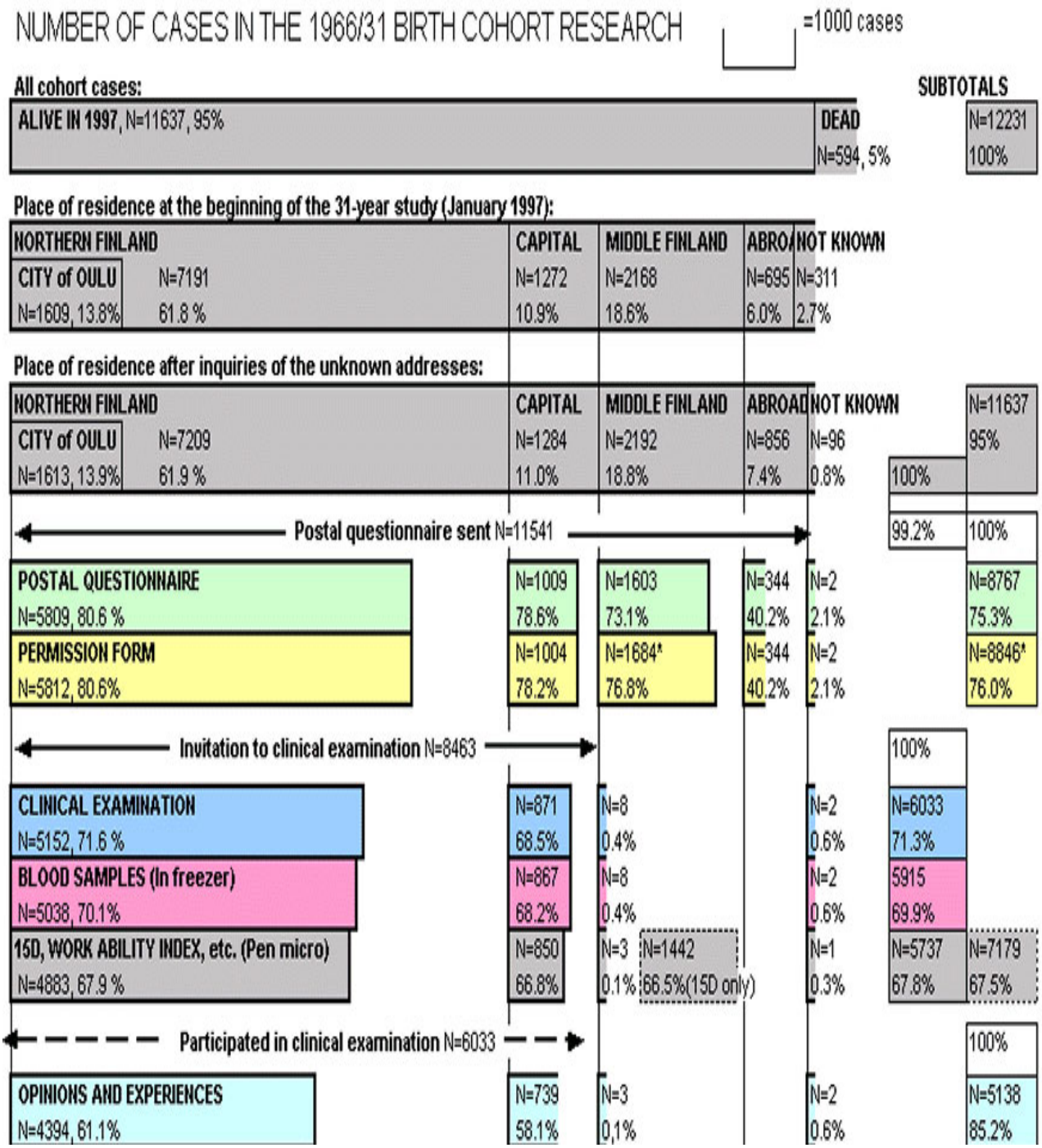


Figure 2.2 Number of participants at 31 years of age and data successfully collected in the NFBC1966. Number of cases in 1966/31 birth cohort research. Available from: **www.oulu.fi/nfbc** [accessed 28th January 2018].

2.1.2.1 Follow-up at 16 years

In 2001, postal questionnaires were sent to both the parents and adolescents. The adolescents' questionnaire included questions on their family, friends, school, mental health, physical health, exercise, behaviour, nutrition, living habits and hobbies. The TAS-20 scale was handed to the participants during the clinical examination sessions. The forms were filled in the clinics while waiting or returned by mail.

During the clinical examination, anthropometric data were measured including weight, height, waist-hip measurements, sitting height, and multiple other measures were taken (spirometry, blood pressure, and pulse rate, bicycle ergometry). Additionally, blood samples were taken and questions about puberty, nutrition, smoking and use of alcohol were also asked. Blood samples including for DNA, leukocyte telomere length (LTL), fasting glucose, insulin, lipids, and selected hormones were successfully collected/analysed from 6795 participants.

Participants were also asked to complete an additional questionnaire concerning eating habits, stress, sexual behaviour, substance use and mental well-being during the clinical examination. The parents' questionnaire included questions on the adolescent's health, development and behaviour. Furthermore, participant’s parents were asked to respond on questions of their marital (family structure) and social status, education, work, health and living habits.

2.1.3 Personalised Medicine for Morbidly Obese Clinical Trial Cohort

The Personalised Medicine for Morbid Obesity (PMMO) is an observational, prospective clinical trial that recruits severely obese adults aged between 18 and 65 years old with BMI >35 kg/m^2.This extreme obesity patient cohort was initiated by Dr Sanne Alsters for her PhD project entitled 'Genetic Analysis of Extreme Obesity' (221), supervised by Professor Alexandra Blakemore. The PMMO project focuses on identifying potential genetic, clinical, behavioural and psychological factors affecting bariatric surgery outcomes. The project was approved by the National Research Ethics Service (NRES) Committee London—West London and Fulham (REC reference 11\LO\0396 and study number 07/Q0411/19).

Patients of Imperial College NHS Weight Centre, Chelsea and Westminster Hospital and Royal Derby Hospital aged 18 between 65 years old with BMI >28 kg/m^2 were recruited by Dr Sanne Alsters, Dr Jennifer Murphy, Dr Olivia Szepietowski, Mr Erdal Ozdemir and trained health professionals, by personal interviews. Written informed consents were obtained from all participants. Anthropometric and clinical data were collected from the patients' hospital records and letters. All clinical data were digitally-stored into an online anonymised central database. Clinical data were obtained by review of electronic medical records from all sites within the hospital network.

2.2 Phenotypic Data

Phenotypic variables involved in this thesis were selected according to the literature, availability, and relevance to each study. The following subsections provide brief descriptions of the phenotypic variables involved. A list of phenotypic variables is presented in Table 2.1.

2.2.1 Adiposity measures

The standard overweight and obesity measures recommended by The International Obesity Task Force (IOTF) (222) and World Health Organization (WHO) consist of BMI cut-off points in adolescent and adults (223). Waist-to-hip ratio (WHR) was also used as another measurement of central obesity in NFBC1966 and NFBC1986.

2.2.1.1 Body mass index (BMI)

Heights and weights were either self-reported or collected from the clinical examinations at each follow-up for all participants. Both measurements (self-reported and clinical examination) were almost identical (Pearson's correlation r=0.98) for NFBC1966 at 31 and 46 years.

BMI ranges were classified according to the WHO International Classification system that defines the following categories: underweight (UW) (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (OW) (25-29.9 kg/m^2) and obese (OB) (≥30 kg/m^2) (224).

For PMMO, all the patients were obese (BMI >35 kg/m^2). Anthropometric data were measured at baseline visit as well as during follow-up visits (to obtain information for weight-loss trajectories following surgery). Weight loss was defined as percentage of initial weight which was lost (%WL), which was calculated by dividing the weight during visit 1 (as baseline), in kilograms, by the weight during each follow-up visit, in kilograms, multiplied by 100. Self-reported baseline and follow-up heights (cm) and weights (kg) were also collected by questionnaires. BMI (kg/m^2) for each participant was calculated by dividing weight (kg) by height^2 (m^2).

BMI change (ΔBMI) was calculated using the following equation:

$$\Delta BMI = BMI_{\text{(post-surgery at 12-month follow-up)}} - BMI_{\text{(baseline)}}$$

In the NFBC1966 cohort, the BMI at 31y and 46y follow-up visits were divided into quartiles with cut-off points, as shown below.

31y follow-up – $Q1_{31y}$: 15.32-21.75 kg/m^2, $Q2_{31y}$: 21.76-23.86 kg/m^2, $Q3_{31y}$: 23.87-26.50 kg/m^2, $Q4_{31y}$: 26.51-54.32 kg/m^2
46y follow-up – $Q1_{46y}$: 16.06-23.57 kg/m^2, $Q2_{46y}$: 23.58-26.15 kg/m^2, $Q3_{46y}$: 26.16-29.41 kg/m^2 and $Q4_{46y}$: 29.42-73.81 kg/m^2

The BMI of the NFBC1986 participants was calculated from height and weight recorded at clinical examinations. Missing data on height and weight from clinical examination were gathered from postal inquiry sent to the adolescents. The BMI quartiles cut-off points were shown as below.

$Q1_{16y}$: 16.79-18.89 kg/m^2, $Q2_{16y}$: 18.90-20.44 kg/m^2, $Q3_{16y}$: 20.45-24.65 kg/m^2, $Q4_{16y}$: 24.66-39.87 kg/m^2.

2.2.1.2 Other obesity measures

In the NFBC1966 and NFBC1986 cohorts, WHR was recorded by teams of trained nurses during the clinical examination at 31y, 46y and 16y follow-up visits. The WHR was calculated according to the guidelines from the WHO for adult's population (NFBC1966) and the International Diabetes Federation (IDF) paediatric criteria for metabolic syndrome for NFBC1986 data. Abdominal or central obesity status can be identified as a WHR > 0.90 in men and > 0.85 in women at 31y and 46y follow-up visits (225). In the NFBC1986 data, the IDF adult cut-off was used to define abdominal obesity due to the waist circumference 90th percentile among girls was 82.0 cm (226). Thus the cut-off points of abdominal obesity for

males and females for NFBC1986 study were 94.0 cm and 80.0 cm respectively (WHR ≥ 85th percentile) (226).

Weight loss was calculated for each PMMO participant as a percentage weight loss (%WL): calculated by dividing the weight during visit 1 (as baseline), in kilograms, by the weight during each follow-up visit, in kilograms, multiplied by 100. Self-reported baseline and follow-up heights (cm) and weights (kg) were also collected by questionnaires.

2.2.2 Psychological measures

2.2.2.1 Alexithymia

The TAS-20 has been used widely as valid and reliable measurement tool for alexithymia (6, 7). The reliability of this alexithymia measure in the Finnish population has been described by Joukamaa *et al.* (2009) (227). Briefly, it has a three-factor structure; 1) Difficulty Identifying Feelings (DIF) which is the inability to communicate feelings to other people and the capacity to identify feelings, and the bodily sensations of emotional arousal 2) Difficulty Describing Feelings (DDF) is the inability to distinguish between feelings and 3) Externally Oriented Thinking (EOT) which is the paucity of fantasy life, concrete speech, and thought closely tied to external events (228). As a standard measure, a person was considered to have clinically-relevant alexithymia if the person's total TAS-20 score ≥ 61 (6). In NFBC1966 and NFBC1986, the TAS-20 scale, together with opinions and experience questionnaire, was handed to the participants of clinical examination in 1997 (31y), 2012 (46y) and in 2001-2002 (16y), as mentioned in previous sections. The forms were filled in the clinics while waiting or returned by mail.

2.2.2.2 Depression

Depression was classified in two ways for the NFBC1966; 1) severity of current depressive symptoms by the 13-item depression subscale (HSCL-13) and 2) clinical depression diagnosis by a physician. Current depressive symptoms were assessed by the 25-item Hopkins Symptom Checklist (HSCL-25) questionnaire at the age of 31 and 46 years in the NFBC1966 participants. The HSCL-25 questionnaire contains the 13-item depression subscale that has been validated as a good instrument for psychiatric cases in Finnish population (152, 229). The current depressive symptoms' score was generated as the sum of the HSCL-13 divided by the number of items answered from the HSCL-25 scale (229). The severity of depressive symptoms was defined using a cut-off score of 1.75 that has consistent prevalence estimates in a general population as clinical diagnosis for depression (230, 231). Self-reported clinical lifetime depression was defined by one item in the questionnaire: whether the study participant had ever been diagnosed as having depression by a physician (No/Yes) at 31-year and 46-year timepoints.

In the PMMO study, the Hospital Anxiety and Depression scale (HADS) (232) was used to measure level of depression and anxiety among severely obese adults at baseline (before surgery) and after surgery (up to 18-24 months).

2.2.2.3 Psychological disorders

For the PMMO cohort, the clinical assessment for psychiatric disorders based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), was conducted by trained psychologists at bariatric surgery centres during visit 1. The psychiatric disorders that were directly assessed included binge eating disorder (BED), clinical depression, bipolar disorder, borderline personality disorder, schizophrenia, learning difficulties, and any history of such disorders.

2.2.3 Other variables

For the PMMO cohort, clinical information such as diabetes mellitus (DM), and other chronic medical illnesses were collected via patient registration forms and recruitment interviews. The socio-demographic variables from the NFBC1966 cohort used in this thesis were marital status, education, employment status and household income level. Marital status was classified as: i) married, ii) cohabiting, iii) unmarried, iv) divorced, or v) widowed. Educational background was divided into four classes: i) basic education or unfinished basic education, ii) basic education with or without vocational training or vocational school or post-secondary school, iii) basic education or matriculation examination with or without polytechnic education iv) basic education or matriculation examination with university degree. The employment status was categorised into three classes: i) employed, ii) unemployed, and iii) "others" (students, retired, and subjects on paternity or maternity leave). The reported family gross income in the previous year was divided into quartiles and used as its income level.

Parent's education and occupation, family type, and annual household income were enquired directly from the parents during the 16-year questionnaire for the NFBC1986 adolescents. Questionnaire items regarding educational background, employment status and annual income for the parents were the same as were used for NFBC1966, thus the same classification system was used for NFBC1986. Family type falls into 4 categories from i) living with both biological parents ii) living with mother or father and with stepfather or stepmother iii) living with single parent iv) living with foster family or partner.

Table 2.1 Phenotypic variables used in current study.

Variable Group	Unit/Categories/Questionnaire item(s)	Note
Adulthood (NFBC1966)		
Age range	31 and 46 years	
Sex	Female and male	
Height	cm	
Weight	kg	
Body mass index (BMI)	Weight (kg)/ height $(m)^2$, quartiles, BMI categories	WHO (2000) (225)
BMI change (ΔBMI)	BMI_{46y} – $BMI_{31y,}$ and subdivided into quartiles with cut-off points: Q1: -19.69-0.57 kg/m^2, Q2: 0.58-2.06 kg/m^2, Q3: 2.07-3.79 kg/m^2, Q4: 3.80-23.24 kg/m^2	
Waist-to-hip-ratio (WHR)	waist circumference (cm)/ hip (cm)	WHO (2011) (224)
Abdominal obesity	WHR >0.90 in men and >0.85 in women	WHO (2000) (225)
Alexithymia (TAS-20 total scores)	Alexithymia; TAS-20 ≥61 Non-alexithymia; TAS-20 ≤60 DIF, DDF and EOT subscales	Bagby *et al.* (1994 a, 1994b) (6, 7)
TAS-20 change (ΔTAS-20)	$TAS\text{-}20_{46y}$ – $TAS\text{-}20_{31y}$	
Depression	Depression symptoms; HSCL-13 subscale ≥1.75 No depression symptoms; HSCL-13 subscale <1.75 Lifetime clinical depression diagnosis by a physician (Yes/ No)	Veijola *et al.* (2003) (229)
Socioeconomic (SES) background	Marital status, education, employment status and household income level	
Pregnancy status	Clinical examinations at 31-year and 46-year follow up	Pregnant women were excluded from the analysis

Variable Group	Unit/Categories/Questionnaire item(s)	Note
Adolescence (NFBC1986)		
Age range	15-16 years	
Sex	Female and male	
Height	cm	
Weight	kg	
Body mass index (BMI)	Weight (kg) / height $(m)^2$, quartiles, BMI categories	Cole *et al.* (2000) (233)
Waist-hip-ratio (WHR)	waist circumference (cm)/ hip (cm)	
Abdominal obesity	WHR ≥ 0.94 in boys and ≥ 0.80 in girls	Jääskeläinen *et al.* (2014) (226).
Alexithymia (TAS-20 total scores)	Alexithymia; TAS-20 ≥61 Non-alexithymia; TAS-20 ≤60 DIF, DDF and EOT subscales	Bagby *et al.* (1994 a, 1994b) (6, 7)
Family's SES	Parent's education and occupation, family type, and annual household income	
Variable Group	**Unit/Categories/Questionnaire item(s)**	**Note**
PMMO		
Age range	21-38, 39-48, 49-55, 56-71 years	
Sex	Female and male	
Ethnicity	British/ Irish, Caribbean/African, Indian/ Pakistani/ Bangladeshi, Mixed background, Any other Caucasian background, Any other Asian background, Any other mixed background and Other	
Height	cm	
Weight	kg	
Body mass index (BMI)	Weight (kg) / height $(m)^2$, quartiles, BMI categories	
Percentage of weight loss (%WL)	Weight (kg) at baseline / Weight (kg) at each follow-up visit multiplied by 100.	
BMI change (ΔBMI)	$BMI_{(post\text{-}surgery\ at\ 12\text{-}month\ follow\text{-}up)} - BMI_{(baseline)}$	
Age of onset obesity (%)	≤10 years old >10 years old	

Alexithymia (TAS-20 total scores)	Alexithymia; TAS-20 ≥61 Non-alexithymia; TAS-20 ≤60 DIF, DDF and EOT subscales	Bagby *et al.* (1994 a, 1994b) (6, 7)
Psychiatric disorders (DSM-5)	Binge eating disorder (BED), clinical depression, bipolar disorder, borderline personality disorder, schizophrenia, and any history of such mental disorders	
Diabetes status	Type 2 diabetes mellitus (T2DM), Insulin-dependent T2DM, Impaired fasting glycaemia, Normal	
Type of bariatric surgery	Roux-en-Y gastric bypass, Gastric band surgery, Vertical sleeve gastrectomy, No surgery	
Depression	HADS- Depression, HADS- Anxiety	Zigmond and Snaith (1983) (232)

2.3 Genetic data

For the NFBC1966 participants who attended the 31-years' clinical examination, blood samples were drawn and DNA was extracted for genotyping (N=5753). Whole blood samples were collected from 5753 participants using vacutainer tubes during the clinical visits, and DNA was subsequently extracted from the blood samples using standard DNA extraction methods. DNA was successfully extracted for 5753 participants from fasted blood samples. Illumina's HumanCNV370-Duo DNA Analysis BeadChip was used for genome-wide genotyping analysis in Chapter 5. The genotyping analysis will be described later in Method's section of Chapter 5. After SNP filtering (SNPs with MAF < 0.05, HWE p<5.7x10-7 or call rate < 0.95 removed) and sample filtering (sample call rate < 0.95, mean heterozygosity < 0.29, multidimensional scaling (MDS) outliers, duplicates, contaminated samples, identity-by-state (IBS) pairwise sharing < 0.20, consent withdrawal or gender mismatch excluded), imputation was done for 1000G Phase 1 reference panel using 364590 SNPs for 5402 participants. DNA extraction was conducted at the Laboratory of National Institute of Health and Well-being in

Helsinki, Finland and SNP genotyping was conducted by laboratory personnel from the Department of Medicine, Imperial College London, UK. IIlumina's HumanCNV370-Duo Analysis BeadChip was used to obtain genome-wide data: this contains tagSNPs derived from the HapMap European-derived (CEU) sample. The genome-wide data included over 318,000 SNPs and about 52,000 markers to target approximately 14,000 copy number variant (CNV) regions, giving a total of over 370,000 markers. The GenomeStudio algorithm was used for calling the genotypes (219).

For NFBC1986 DNA was extracted from 6266 participants who attended clinical examination at the 16y follow-up. Genotyping was performed at Imperial College London, UK or in the Pasteur Institute Laboratories, Lille, France. The genotyping success rate for all SNPs was >95.0% and none of the SNPs deviated from Hardy-Weinberg equilibrium (all p-values>0.0001). A total of 3834 samples were genotyped using Illumina HumanOmniExpressExome-8v1.2 platform and BeadStudio calling algorithm. After SNP filtering (SNPs with HWE $p<1x10^{-4}$, call rate < 0.99 removed) and sample filtering (call rate < 0.95, mean heterozygosity < 0.305, IBS pairwise sharing < 0.2, gender mismatch, duplicate samples or consent withdrawal), genotype data was available for 3743 adolescents. This includes a selected set of 372 individuals exposed to gestational diabetes, gestational hypertensive disorders and preterm birth, and the rest is a random sample. Imputation was done based on 889119 SNPs for 1000G Phase 3 imputation panel.

Sample and SNP quality control (QC) for NFBC1966 and NFBC1986 was conducted by Nikman Adli Nor Hashim as part of his PhD study at Imperial College London, United Kingdom from Professor Alexandra Blakemore's research group.

2.4 Data analysis strategy

This section describes overall statistical methods used in this dissertation and more details on statistical modelling and approach used are described in each result chapter (Chapter 3, Chapter 4, Chapter 5 and Chapter 6). The analytical strategy and methods applied are standard statistical approaches for genetic, epidemiological and clinical data. Appropriate statistical methods conducted in this thesis are to explore the nature and quality of the data (e.g. distributions, outliers, errors), potential confounders to consider, unadjusted associations, impact of stratification (e.g. by sex) and then proceeding to test the research hypothesis, and further answering research questions as per set in the data analytical framework (Figure 1.4). More sophisticated multivariable and longitudinal data analyses are to explore the impact of covariates and assess the independent (i.e. adjusted) associations between the key exposures and the outcomes, and potential role of confounders to serve the purpose and aims of the study. The relevant analyses were conducted step by step.

2.4.1 Phenotypic data

Overall data analyses approaches are described here but study-specific analytical approaches are described further in relevant chapters. For all analyses, the Statistical Package for Social Sciences software (SPSS for Windows, version 22.0, 2004, Chicago, IL, USA) was used. Student's t-test (t) (paired t-test for repeated, non-independent measures), analysis of covariance (ANCOVA) and Pearson's chi-squared test (χ^2) were appropriately used to compare continuous and categorical variables, at cross-sectional and longitudinal levels. Bonferroni adjustment was used for multiple testing correction of main effects comparisons and interactions in ANCOVA models.

The selected variables from NFBC1966 and NFBC1986 datasets were examined for distribution to determine for any possible errors and outliers. The SPSS procedure 'Descriptive

Statistics' in version 20.0 (Armonk, NY: IBM Corp.) was used to explore the nature and quality of the data (e.g. distributions, outliers, errors).

For all continuous variables, means and standard deviations (SD) were presented while percentage distributions for each category were given for the categorical variables. Once the data were cleaned, the characteristics of the study population were plotted on scatter or histogram plots. For each analysis, dependent and independent variables were specified, and covariates were selected as adjustments in the association analyses. Covariate selection was based on literature, empirical experiences and association/ descriptive analyses. Scatter plots were also used to look for possible linear or non-linear relationships between dependent and independent variables.

The Pearson correlation coefficient (r) was used to check the potential collinearity between continuous variables. The correlation coefficient ranges from +1 to -1, where a score of ±1 shows a perfect positive or negative linear relationship and a score of zero shows no correlation between the two variables.

Once the selection of variables to be included was finalised, the final model was used to test the inferred associative relationship using standard procedures: either analysis of variance (ANOVA), analysis of covariance (ANCOVA), or multiple linear regression model as appropriate. The results were interpreted by looking at the point estimates using their p-values and 95% confidence intervals (CI) to assess the level of significance. A significant result was considered if the p-value was <0.05 and the 95% CI did not cross zero when comparing the difference between the mean values of comparison groups. The t- and F-test statistics were also considered to examine the significance of the model itself (p-value <0.05 was considered significant).

Normality, linearity, homoscedasticity, and multi-collinearity were assessed to ensure that assumptions of regression models were met. The underlying assumptions for the multiple linear regression model were assessed using P-P, histogram and scatter plots (ZPRED vs ZRESID). The linearity between the independent variables and the dependent variable was assessed using scatter and Q-Q plots. For multicollinearity, collinearity statistics were performed and absence of multi-collinearity was observed in the current analysis (Tolerance > 0.1 and VIF < 2) (234).

Adjusted R^2 with ±SEM (standard error of measurement) was calculated to identify proportion of variability in the outcome (dependent) variables that is explained by predictors or independent variables in multiple regression models. A stepwise multiple linear regression analysis was conducted, adjusted for sex, education level, annual income level, marital status and type of employment to predict BMI outcome over time in the NFBC1966. During the first step of the multiple linear regression procedure, sex and SES factors; education level, annual income level, marital status and type of employment were entered into the model. In the second step of the BMI prediction model, TAS-20 score and HSCL-13 score were entered, to explore which variables were significant predictors of BMI difference at the age of 46 years.

For analysis of the PMMO cohort, age, sex, and ethnicity were used as standard covariates in multiple linear regression models, as described in Chapter 5. The type of surgery was also included as an additional predictor in post-surgery regression models. The residuals were normally distributed and there was homoscedasticity in both pre- and post-surgery multiple liner regression analysis.

2.4.2 Genotypic data analyses

A detailed description of genome-wide association studies (GWAS) has been published by Bush and Moore (2012) (235). Briefly, two genetic association analyses on TAS-20 scores were conducted separately in the NFBC1966 and NFBC1986 cohorts. Additionally, genetic

association analyses were also conducted for current depressive symptoms (HSCL-13 scores) as continuous traits using the PLINK 1.07 software package (236). Genotype frequencies and possible departures from HWE in the study population were calculated with *missing*, *freq* and *hardy* functions in PLINK v1.07 (236, 237).

Linkage disequilibrium (LD) structures of the genetic regions of interest were visualised with Haploview v3(238). For significant variants or SNPs associated with TAS-20 or HSCL-13 scores, the SNPsnap web-based tool was used to match and annotate SNPs to European 1000 Genome Project reference (1000G Phase 3) (239). For 'functional mapping and annotation of genetic associations, FUMA (240) and MAGMA (1) were used for post-GWAS interpretation. Using the FUMA platform, Manhattan plots, QQ plots and regional association plots were created for the TAS-20 and HSCL-13 score GWAS datasets.

Pairwise linkage disequilibrium (LD) statistics (r^2) for SNPs located within same chromosome were assessed using the FUMA(240) and SNiPA platform (241). Manhattan plots were created to visualise top hits from the GWAS with the X-axis, showing the genomic co-ordinates, and the Y-axis showing the negative logarithm (denoted by log-10(P)) of the associated SNP p-value. Similarly, association plots were used to visualise a limited section of the genome, with X-axis showing the position of the SNP of interest on the chromosome, and the Y-axis showing the negative logarithm of the associated SNP p-value.

CHAPTER 3 Association between Alexithymia and Obesity in the Northern Finnish Adolescent and Adult Population

3.1 INTRODUCTION

This chapter describes an exploration of the relationships between emotion processing deficits as measured by TAS-20 scores, and adiposity in an unselected, general European population.

Recent systematic reviews and meta-analysis studies investigating alexithymia's role in obesity and eating disorder have shown that individuals with either obesity or eating disorders (ED) exhibited a higher prevalence of alexithymia compared to non-obese or non-ED controls(56, 58). Availability of the 20-item or 26-item Toronto Alexithymia Scale (TAS-20 and TAS-26) was used as one of the eligibility criteria of the study selection. Their findings, however, included contradictory results about associations of body mass index (BMI) with total TAS-20 scores, and sex differences in alexithymia prevalence among obese and ED populations. Westwood *et al.* (2017) (58) report that BMI may be associated with alexithymia in anorexia nervosa (AN). In her meta-analysis review, Westwood *et al.* (2017) (58) found positive BMI difference between alexithymia and non-alexithymia groups among anorexic patients. In contrast, Fernandes *et al.* (2017)(242) report no significant association between BMI and the total TAS-20 score in three studies of participants with obesity; 1) patients seeking weight loss surgery and already submitted to treatment (243) 2) patients undergoing conservative weight loss intervention (244) and 3) diet-seeking obese patients admitted to a medical ward.

There are some studies also in non-clinical populations. The results reported from different populations are overall inconsistent on the relationship between alexithymia and obesity (22, 49, 62, 245, 246). This may be due to the complexities of the emotion regulation process in obesity itself. There are different strategies to deal with emotions

and it remains difficult to determine whether emotional processing deficits are contribute to, or are attributable to obesity. Additionally, there are cross-cultural differences in coping mechanisms (247) and different societies and cultures have their own perspectives on obesity. Heterogeneous study samples, study design and sample sizes may also contribute to the inconsistencies in findings about the relationship between obesity and alexithymia. Thus, it is clear that the relationship between BMI and alexithymia needs further exploration in larger, unselected populations. Well-phenotyped large longitudinal cohorts are excellent for this purpose. To date, no information on the relationships between alexithymia, BMI and change in BMI over the life-course in general populations has been reported.

Here, I present results from association analyses conducted on alexithymia measures (TAS-20 score and its subscales) together with body mass index (BMI, kg/m^2) and waist-hip ratio (WHR, cm) at adolescence and in adulthood, in the NFBC1986 and NFBC1966 birth cohorts (described in detail in Chapter 2). I also intended to test whether TAS-20 score and its subscales can predict BMI at later age among adults in the NFBC1966.

In NFBC1986, alexithymia was assessed at age 16, and assessment was at the ages of 31 and 46 in the NFBC1966. In epidemiological and genetic association studies, BMI and WHR are often used to obesity research, because of the ease of measurement in large samples coupled with the well-established associations with metabolic and health outcomes (248).

Figure 3.1 provides the framework for subject selection and the final numbers of participants from both NFBC1966 and NFBC1986 cohorts that were eligible for the analysis. The demographics of alexithymia in both cohorts (NFBC1986 and

NFBC1966) are presented in Section 3.3.1 and Section 3.3.2, followed by assessment of the relationships between alexithymia and obesity measures at each of the timepoints in Section 3.3. A longitudinal analysis of change of alexithymia and obesity over the 15-year period between ages 31 and 46 years in the NFBC1966 is presented in Section 3.3.4. Finally, Section 3.4 gives a brief summary and discussion of the findings.

3.1.1 Aims of the study

i. To explore the relationships between TAS-20 scores and obesity measures (BMI and WHR) in the NFBC1966 and NFBC1986 cohorts.

ii. To carry out a longitudinal analysis of TAS-20 scores and obesity in the NFBC1966 cohort.

iii. To predict BMI outcome at later age (46-years) in the longitudinal analysis of NFBC1966.

I aimed to address the following research questions:

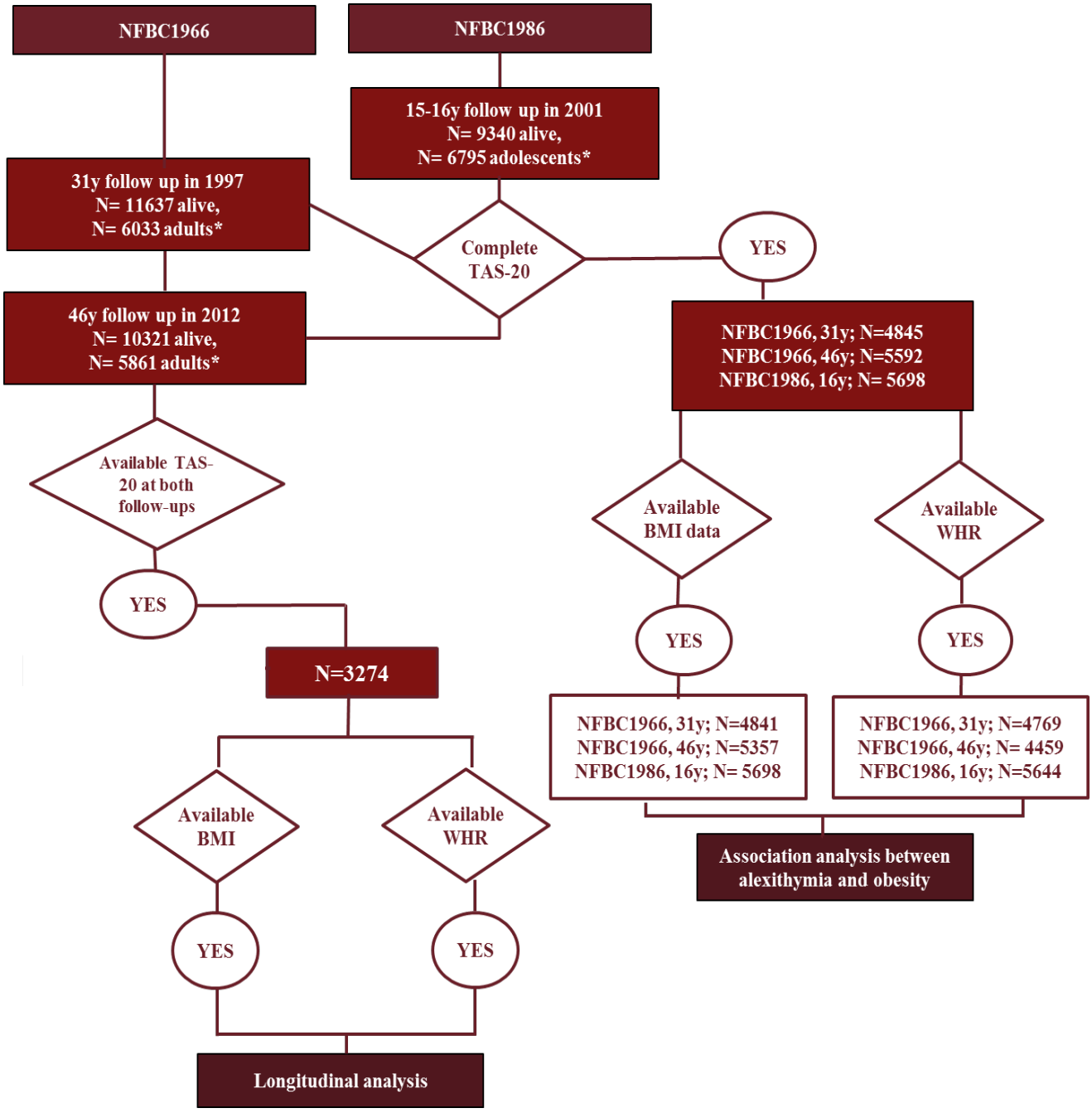
i. Are there any associations between alexithymia and obesity in these two unselected European general population cohorts?

ii. Is there any relationship between TAS-20 score change and BMI change?

3.1.2 Null hypotheses

i. There are no associations between BMI and TAS-20 scores in general adult and adolescent populations, analysed cross-sectionally.

ii. There is no relationship between change in BMI over time and TAS-20 scores.



*returned postal questionnaires and participated clinical examination

Figure 3.1 Flow chart on formation of present sample over the life-course and statistical analysis framework.

3.2 METHODS

All participants who gave consent, completed TAS-20 questionnaire via postal inquiry, with available BMI or WHR data, and were not pregnant at the age of 16 (NFBC1986), 31 and 46 (NFBC1966) were included in the association analyses between alexithymia and adiposity measures (Figure 3.2). Pregnant women at 16y (n=6), 31y (n=216), and 46y (n=1) timepoints were identified and removed, because BMI classifications for pregnant women are difficult to interpret.

Phenotypic variables selected for the analyses in this chapter were the TAS-20 total scores and its subscales (DIF, DDF and EOT) as main outcomes or dependent continuous variables, analysed against obesity measures; BMI and WHR. Sex and socioeconomic status (SES) were included as covariates and tested against exposures and outcomes in the association analysis. BMI, based on clinical examination, was calculated as weight (in kilograms) divided by height (in metres) squared (kg/m^2) and BMI groups were defined according to the WHO International Classification system (233).

As shown in Figure 3.2, BMI was subdivided into quartiles at each timepoint for NFBC1966 and NFBC1986; $Q1_{31y}$: 15.32-21.75 kg/m^2, $Q2_{31y}$: 21.76-23.86 kg/m^2, $Q3_{31y}$: 23.87-26.50 kg/m^2, $Q4_{31y}$: 26.51-54.32 kg/m^2, $Q1_{46y}$: 16.06-23.57 kg/m^2, $Q2_{46y}$: 23.58-26.15 kg/m^2, $Q3_{46y}$: 26.16-29.41 kg/m^2 and $Q4_{46y}$: 29.42-73.81 kg/m^2. For NFBC1986, BMI quartiles cut-off points were $Q1_{16y}$: 16.79-18.89 kg/m^2, $Q2_{16y}$: 18.90-20.44 kg/m^2, $Q3_{16y}$: 20.45-24.65 kg/m^2, $Q4_{16y}$: 24.66-39.87 kg/m^2. Quartiles analysis shows the spread of each datasets at different time points in the birth cohorts.

Student's t-test (t), analysis of covariance (ANCOVA) and Pearson's chi-squared test (χ^2) were appropriately used to compare continuous and categorical variables, in cross-sectional and longitudinal analyses. Bonferroni adjustment was used for multiple testing correction of main

effects comparisons and interactions in ANCOVA models. Sex differences were determined either by one-way ANOVA or independent t-test between continuous dependent variables. All assumptions have been tested and met for statistical analyses.

For the longitudinal analysis of TAS-20 and BMI change over the 15-year period (between when the participant were 31 and 46 years old) in the NFBC1966, 3274 (55.9%; males, n= 1396, females, n= 1878) of the total 46-year participants who attended the clinical examinations (n=5861) were analysed. The association between TAS-20 (or ΔTAS-20) and BMI (or ΔBMI) at 31 years (baseline) and 46 years (timepoint) was assessed using Pearson's correlation (r), and ANCOVA. BMI change was calculated as $\Delta BMI = BMI_{46y} - BMI_{31y}$. Data were subdivided into quartiles (Figure 3.3) with cut-off points for Q1: -19.69-0.57 kg/m^2, Q2: 0.58-2.06 kg/m^2, Q3: 2.07-3.79 kg/m^2, Q4: 3.80-23.24 kg/m^2.

A multiple regression model was conducted to further predict BMI outcome at age of 46 years by the TAS-20 total scores (and its subscales) at age of 31 years. Sex, marital status, education level, employment, and annual income were used as standard covariates. The clinical cut-off for alexithymia status (TAS-20 ≥61) was used as independent variable to predict BMI outcome in the longitudinal analysis. Adjusted R^2 with ±SEM (standard error of measurement) were calculated and presented to ascertain the proportion of variability (total variance) in the outcome (dependent) variables that is explained by predictors or independent variables in multiple regression models.

3.3 RESULTS

3.3.1 Alexithymia demographics in NFBC1986

At the NFBC1986 16-year timepoint (in 2001-2002), the adolescents and their parents filled in postal questionnaires (response rates 77.9%, n=7182 and 74.5%, n=6866, respectively). Anthropometric measurements (height in centimetres, weight in kilograms to one decimal place, waist circumference in centimetres) were obtained from 6795 (73.7%) 15-16 year old adolescents at a clinical examination, conducted by trained nurses. After exclusion of ineligible subjects (those without consent forms, not attending clinical examination, not completing TAS-20 questionnaire and missing BMI data), 2781 boys and 2922 girls were included in the analyses.

Indicators of socio-economic status (SES), such as parent's education, occupation, and family's annual gross income were taken from postal questionnaire to the participant's parents, and family type (intact; living with both biological parents and non-intact; living either with one biological parent and step father or step mother, single parent, adopted family and partner) was from the adolescent's postal inquiry. The description of SES is described in Chapter 2 (Material and Methods).

Table 3.1 shows the prevalence of alexithymia at 15-16 years of age (n=428, 7.5%). There was a sex difference; alexithymia was more prevalent in girls (8.5%) compared to boys (6.5%) ($p=0.007$). There was a significant sex difference between alexithymia and non-alexithymia ($p=0.007$) groups. Also, there was a significant sex difference on mean total TAS-20 score ($p<0.001$). Higher alexithymia score in this adolescent cohort was associated with lower parental education background, broken family, lower family annual income, and having an unemployed father.

Table 3.1 Demographics of 15-16-year-old participants in the NFBC1986 with available data (n=5698). Data presented as mean (±SD) for continuous variables and as a percentage for categorical variables.

	NFBC1986	**Non-Alexithymia**	**Alexithymia**	***P-value**	**[¥]P-value**
N (%)	5698 (100)	5270 (92.5)	428 (7.5)		
Male/Female (%)	2781/2922 (48.8/51.2)	2600/2674 (93.5/91.5)	181/248 (6.5/8.5)	**0.007**	**<0.001**
BMI (kg/m^2)	21.1 (±3.4)	21.1 (±3.4)	21.5 (±3.6)	**0.02**	0.193
WHR (cm)	0.80 (±0.1)	0.79 (±0.1)	0.80 (±0.1)	**0.02**	**<0.001**
BMI Groups(233)				0.133	**0.015**
Underweight	245 (4.3)	232 (4.4)	13 (3)		
Normal	4708 (82.6)	4362 (82.8)	346 (80.8)		
Overweight	509 (8.9)	464 (8.8)	45 (10.5)		
Obese	236 (4.1)	212 (4)	24 (5.6)		
TAS-20	47.8 (±8.7)	47 (±8)	64 (±3)		-
DIF	14.9 (±4.8)	14.2 (±4.2)	23.1 (±3.4)		
DDF	11.0 (±3.4)	10.5 (±3.0)	16.9 (±2.3)		
EOT	21.9 (±4.2)	21.7 (±4.1)	24.5 (±3.5)		
Mother's Education					
1 - low	211 (4.3)	189 (2.4)	22 (3.8)	**0.002**	**<0.001**
2	3039 (63.8)	2796 (63.3)	243 (69.8)		
3	1144 (23.5)	1070 (23.7)	74 (20.3)		
4 - high	495 (10.3)	473 (10.6)	22 (6)		
Father's Education				0.162	**<0.001**
1 - low	186 (4)	166 (3.8)	20 (5.8)		
2	2941 (62)	2719 (62.3)	222 (64.2)		
3	1124 (24)	1047 (24)	77 (22.3)		
4 - high	457 (10)	430 (9.9)	27 (7.8)		
Family type				**0.001**	**<0.001**
Nuclear or intact	4173 (78.4)	3885 (78.9)	288 (71.8)		
Separated or divorced	1150 (21.6)	1037(21.1)	113 (28.2)		
Family gross income				**0.026**	**<0.001**
1 - low	923 (23.4)	835 (22.8)	88 (30.7)		
2	917 (23.2)	857 (23.4)	60 (20.9)		
3	829 (21)	773 (21.1)	56 (19.5)		
4 - high	1280 (32.4)	1197 (32.7)	83 (28.9)		
Mother's Occupation				0.391	0.503
Employed	3743 (80.5)	3467 (80.6)	276 (79.1)		
Unemployed	590 (12.7)	545 (12.7)	45 (12.9)		
[#]Others	319 (6.9)	291 (6.8)	28 (8)		
Father's Occupation				0.105	**0.032**
Employed	3940 (87.9)	3661 (88.2)	279 (84.5)		
Unemployed	271 (6)	243 (5.9)	28 (8.5)		
[#]Others	269 (6.1)	246 (5.9)	23 (7)		

*Comparison between non-alexithymia and alexithymia groups. [¥]TAS-20 score was used as continuous dependant variable in ANOVA one-way analysis on BMI and SES factor and Pearson's correlation to test difference in continuous adiposity measures (WHR and BMI). [#]Students, retired, and subjects on paternity or maternity leave.

3.3.2 Alexithymia demographics in NFBC1966

From the whole NFBC1966, 4841 (male: n= 2277, female: n=2564) and 5404 (male: 2382, female: n=3022) participants responded to both TAS-20 and socio-demographic questionnaires, of which 6.9% and 6.5% passed the threshold for alexithymia (TAS-20 ≥61) at age of 31 and 46, respectively. As shown in Table 3.2 and 3, 208 males (4.3%) and 126 females (2.6%) had alexithymia status at 31-year timepoint and 210 males (3.9%) and 141 females (2.6%) had alexithymia status at 46-year timepoint.

A total of 3274 participants had available BMI and TAS-20 data at both timepoints (1997 and 2012). The mean BMI at age 31 years and 46 years was 24.3 (±4.25) kg/m^2 and 26.7 (±4.94) kg/m^2, respectively. The study subject characteristics by sex difference at both timepoints (31 and 46 years) in the NFBC1966 are presented in Table 3.4. Sex differences were observed in all of the subscales across the TAS-20 at 31 years of age (DIF_{31y}: p= 0.017; DDF_{31y}: p <0.001; EOT_{31y}: p<0.001). At 46 years of age, the DDF subscale lost the sex significant difference but for DIF and EOT subscales, the sex difference remained (DIF_{46y}: p= 0.005; EOT_{46y}: p<0.001).

There was a minor but statistically significant difference between the alexithymia prevalence between 31 years (n=199 or 6.1%) and 46 years (n=206 or 6.3%) ($\chi_{(1)}$= 310.32, p<0.001). The proportion of individuals falling into various levels of socio-demographic characteristics was different between alexithymia and non-alexithymia at both timepoints such that individuals with alexithymia often had 9-year basic education (31y: $\chi_{(3)}$= 108.32, p<0.001, 46y: $\chi_{(3)}$= 81.70, p<0.001) and lower income (31y: $\chi_{(3)}$= 34.04, p<0.001, 46y: $\chi_{(3)}$= 36.52, p<0.001). Alexithymic individuals were more likely to be single at 31-year ($\chi_{(4)}$=79.08, p<0.001) and 46-year timepoints ($\chi_{(4)}$=29.65, p<0.001).

Table 3.2 Demographics of NFBC1966 participants at 31-year timepoint with available data. Data are presented as mean (±SD) for continuous (TAS-20 scores and BMI) variables and as a percentage for categorical variables. The TAS-20 score has a three-factor structure, consisting of Difficulty Identifying Feelings (DIF), Difficulty Describing Feelings (DDF), and Externally Oriented Thinking (EOT). BMI ranges were classified according to the WHO International Classification system.

Group	**NFBC1966 N= 4841**	**Non-Alexithymia N= 4507**	**Alexithymia N= 334**	***P-value**
Male/Female (%)	2277/2564 (47.0/53.0)	2069/2438 (45.9/54.1)	208/126 (62.3/37.7)	**<0.001**
BMI, kg/m^2 (±SD)	24.6 (±4.3)	24.5 (±4.2)	25.5 (±5.1)	**<0.001**
WHR, cm (±SD)	0.86 (±0.1)	0.85 (±0.1)	0.89 (±0.1)	**<0.001**
TAS-20 scores				
Total TAS-20	44.0 (±10.2)	42.7 (±8.8)	65.5 (±4.1)	**<0.001**
DIF	13.4 (±4.7)	12.8 (±4.1)	22.8 (±3.9)	**<0.001**
DDF	10.8 (±3.9)	10.4 (±3.5)	17.7 (±2.3)	**<0.001**
EOT	19.7 (±4.7)	19.5 (±4.5)	25.1 (±3.5)	**<0.001**
Marital status (%)				**<0.001**
Married	2265 (47.1)	2152 (48.1)	113 (34.1)	
Co-habiting	1179 (24.5)	1116 (24.9)	63 (19.0)	
Single	1166 (24.3)	1019 (22.8)	147 (44.4)	
Divorced or widowed	196 (4.1)	188 (4.2)	8 (2.4)	
Education (%)				**<0.001**
1 - low	22 (0.3)	12 (0.2)	10 (3.0)	
2	2526 (51.1)	2292 (48.4)	234 (70.7)	
3	1760 (37.6)	1686 (39.2)	74 (22.4)	
4 - high	511 (11.1)	498 (12.2)	13 (3.9)	
Income (%)				**<0.001**
1 - low	1120 (25.7)	1008 (24.7)	112 (40.0)	
2	1186 (27.2)	1120 (27.5)	66 (23.7)	
3	1000 (23.0)	945 (23.2)	55 (19.7)	
4 - high	1049 (24.1)	1003 (24.6)	46 (16.5)	
Occupation (%)				**<0.001**
Employed	3327 (69.3)	3128 (70.0)	199 (60.0)	
Unemployed	816 (17.0)	733 (16.4)	83 (25.0)	
Others	655 (13.7)	605 (13.5)	50 (15.0)	
BMI group (%)				**0.004**
Underweight	117 (2.4)	106 (2.4)	11 (3.3)	
Normal	2824 (58.3)	2657 (59.0)	167 (50.9)	
Overweight	1469 (30.3)	1356 (30.1)	113 (33.8)	
Obese	431 (8.9)	388 (8.6)	43 (12.9)	

*Comparisons between alexithymia groups used 2-sided independent t-test for continuous variables and a $\chi 2$ test between categorical variables.

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Table 3.3 Characteristics of NFBC1966 participants at 46 year timepoint with available data. Data are presented as mean (±SD) for continuous variables and as a percentage for categorical variable. The TAS-20 score has a three-factor structure, consisting of Difficulty Identifying Feelings (DIF), Difficulty Describing Feelings (DDF), and Externally Oriented Thinking (EOT). BMI ranges were classified according to the WHO International Classification system.

Group	**NFBC1966 at 46-year N = 5404**	**Non-Alexithymia N = 5053**	**Alexithymia N = 351**	**P-value**
Male/Female (%)	2382/3022 (44.0/56.0)	2172/2881 (43.0/57.0)	210/141 (60.0/40.0)	**<0.001**
BMI, kg/m^2 (±SD)	26.8 (±4.9)	26.7 (±4.8)	28.2 (±6.0)	**<0.001**
WHR, cm (±SD)	0.91 (±0.1)	0.91 (±0.1)	0.95 (±0.1)	**<0.001**
TAS-20 scores				
Total TAS-20	44.1 (±10.1)	42.6 (±8.6)	65.5 (±4.4)	**<0.001**
DIF	13.3 (±4.7)	12.7 (±4.0)	22.7 (±3.7)	**<0.001**
DDF	10.8 (±3.7)	10.3 (±3.3)	17.9 (±2.2)	**<0.001**
EOT	20.1 (±4.4)	19.7 (±4.3)	24.9 (±3.1)	**<0.001**
Marital status (%)				**<0.001**
Married	3141 (60.0)	2959 (60.5)	182 (53.2)	
Co-habiting	972 (18.6)	911 (18.6)	61 (17.8)	
Single	594 (11.4)	525 (10.7)	69 (20.2)	
Divorced or widowed	526 (10.1)	496 (10.1)	30 (8.8)	
Education (%)				**<0.001**
1 – low	80 (1.5)	69 (1.4)	10 (3.2)	
2	1703 (32.5)	1523 (31.1)	180 (52.5)	
3	1150 (21.9)	1086 (22.1)	64 (18.7)	
4 – high	2313 (44.1)	2225 (45.4)	88 (25.6)	
Income (%)				**<0.001**
1 – low	1066 (22.4)	971 (21.8)	95 (31.8)	
2	1227 (25.8)	1144 (25.6)	83 (27.8)	
3	1084 (22.8)	1008 (22.6)	76 (25.4)	
4 – high	1383 (29.1)	1338 (30.0)	45 (15.1)	
Occupation (%)				**<0.001**
Employed	4619 (89.0)	4365 (89.9)	254 (75.8)	
Unemployed	292 (5.6)	249 (5.1)	43 (12.8)	
Others	279 (5.4)	241 (5.0)	38 (11.3)	
BMI group (%)				**<0.001**
Underweight	35 (6.5)	32 (0.6)	3 (0.9)	
Normal	2094 (38.7)	1992 (39.4)	102 (29.1)	
Overweight	2144 (39.7)	2009 (39.8)	135 (38.5)	
Obese	1131 (20.9)	1020 (20.2)	111 (31.6)	

*Comparisons between alexithymia groups used 2-sided independent t-test for continuous variables and a $\chi 2$ test between categorical variables.

Table 3.4 Comparison of NFBC1966 participants by sex (n=3274) at both baseline (31years) and timepoint (46 years). Texts marked in bold indicate variables and numbers that are significant on the 95% confidence limit. Overall column in the table shows the sample size and the mean (±SD) for variables in the longitudinal dataset.

Variables	**31years**				**46years**				***P-value**
	Males	**Females**	**¥P-value**	**Overall**	**Males**	**Females**	**¥P-value**	**Overall**	
N(%)	1396(42.6)	1878(57.4)		3274	1396(42.6)	1878(57.4)		3274	
BMI	25.1(±3.4)	24.0(±4.6)	**<0.001**	24.5(±4.1)	27.1(±4.1)	26.7(±5.5)	**0.002**	26.8(±4.9)	**<0.001**
WHR	0.91(±0.1)	0.81(±0.1)	**<0.001**	0.816(±0.1)	0.98(±0.1)	0.87(±0.1)	**<0.001**	0.92(±0.1)	**<0.001**
Total TAS-20	46.3(±9.7)	42.2(±10.0)	**<0.001**	43.9(±10.1)	46.8(±9.6)	42.2(±9.7)	**<0.001**	44.1(±9.9)	0.205
DIF	13.1(±4.5)	13.7(±4.7)	**<0.001**	13.4(±4.6)	13.3(±4.6)	13.2(±4.6)	0.349	13.2(±4.6)	**0.015**
DDF	11.7(±3.9)	10.2(±3.7)	**<0.001**	10.8(±3.9)	11.7(±3.7)	10.1(±3.6)	**<0.001**	10.8(±3.7)	0.372
EOT	21.5(±4.4)	18.4(±4.4)	**<0.001**	19.7(±4.6)	21.8(±3.9)	18.9(±4.2)	**<0.001**	20.1(±4.3)	**0.005**
Alexithymia cases, n(%)	110(7.9)	89(4.7)	**<0.001**	199(6.1)	114(8.2)	92(4.9)	**<0.001**	206(6.3)	**<0.001**

Data is presented as mean (±SD) for continuous variables and as a percentage for categorical variables. **¥**Comparisons between genders used 2-sided independent t-test for continuous variables and a $\chi 2$ test between categorical variables. *Overall timepoint comparisons used 2-sided paired t-test and $\chi 2$ tests.

3.3.3 Relationship between alexithymia and obesity measures among adolescence in NFBC1986

As can be seen in the table above (Table 3.1), differences were observed between people with alexithymia and those without, in BMI groups and WHR at the 16 year timepoint, in the NFBC1986 cohort. There was a weak positive correlation between TAS-20 score and WHR at 16 years of age (16years; $r_{(5644)}$=0.08, p<0.001). Association between TAS-20 score and BMI quartiles was observed at age of 16 in the NFBC1986 (p=0.005), adjusted for sex (Figure 3.2). The association, however, was not any more significant when adjusted for parent's SES.

In a separate analysis, when TAS-20 score was analysed as a categorical variable (alexithymia vs non alexithymia), a significant association was found between alexithymia and BMI quartiles at age of 16 in the NFBC1986 (p=0.014). There was also significant association between alexithymia (as a categorical trait, TAS-20≥ 61) and BMI when adjusted for sex ($F_{(1,5695)}$= 5.19, p=0.02).

Alexithymic participants (n=428) had nominally higher mean BMI, 21.50 (±3.6) kg/m^2 compared to non-alexithymic (n=5274) participants, 21.11 (±3.4) kg/m^2 ($t_{(5696)}$= 2.30, p= 0.02). Pearson's correlation showed no correlation between TAS-20 and BMI (r=0.02, p=0.208). Conversely, BMI groups and WHR were significantly associated with TAS-20 total scores (BMI groups; global p=0.015, WHR; p<0.001).

The TAS-20 mean score for males was 48.45 (±8.2) and, 47.25 (±9.2) for female. The mean WHR for males was 0.82 (±0.05) cm and, 0.77 cm (±0.05) for females. The mean BMI for males and females was 21.11 kg/m^2 and 21.17 kg/m^2, respectively.

3.3.4 Relationship between alexithymia and obesity measures among adults in NFBC1966

In adults, the mean TAS-20 total scores was significantly different between BMI quartiles, in NFBC1966 (31y; p=0.02, 46y; p=0.002) after controlling for sex and SES at 31-year timepoint (Figure 3.2). As presented in the later longitudinal analysis section, there were also sex differences in ΔBMI and ΔWHR but not in ΔTAS-20 in the adult cohort. At 31 and 46 years of age, there were significant sex differences at the population level in TAS-20 scores ($p<0.001$) and obesity measures; BMI and WHR ($p<0.001$).

There was also significant association between alexithymia as a clinically-relevant binary trait, (TAS-20≥ 61) and BMI when adjusted for sex (31y: $F_{(1,4838)}= 12.05$, $p= 0.001$, 46y: $F_{(1,3271)}= 10.09$, $p= 0.002$). Alexithymic participants had higher mean BMI compared to non-alexithymic participants by 1.04 (±0.24) kg/m2 and 1.49 (±0.27) kg/m2, respectively (31y: $t_{(5038)}= 4.33$, $p< 0.0001$, 46y: $t_{(5395)}= 1.49$, $p< 0.0001$).

Pearson's correlation analysis revealed weak positive correlations between TAS-20 score and BMI at 31 and 46 years of age (31y: $r_{(4841)}=0.10$, $p<0.001$, 46y: $r_{(5357)}=0.11$, $p<0.001$). There was also positive correlation between TAS-20 score and WHR (31y: $r_{(4769)}=0.18$, $p<0.001$, 46y: $r_{(4564)}=0.22$, $p<0.001$). Mean differences, corroborating the correlation, indicated that the alexithymia score increased, on average, by roughly one point for every quartile increase in BMI.

BMI at 31 and 46 years was associated with all the TAS-20 subscales although correlation coefficients were quite low (DDF_{31y} $r_{(4841)}=0.056$; $p=0.001$, DDF_{46y} $r_{(5404)}=0.063$; $p<0.001$, DIF_{31y} $r_{(4841)}=0.039$; $p=0.024$, DIF_{46y} $r_{(5404)}=0.087$; $p<0.001$, EOT_{31y} $r_{(4841)}=0.107$; $p<0.001$, EOT_{46y} $r_{(5404)}=0.102$; $p<0.001$). This association between TAS-20 subscales and BMI was not observed at the age of 15-16 years in the NFBC1986 cohort.

The TAS-20 mean differences against BMI groups (WHO classifications) at 31 and 46 years were dependent on the differences manifested in the EOT subscale, adjusted for sex, and SES (EOT_{31y}: $F_{(3,4287)}$= 5.48, p=0.001; EOT_{46y}: $F_{(3,4630)}$= 5.22, p=0.001). Bonferoni's post-hoc assessment indicated that, at 31 years old, both males and females who were obese had higher mean EOT subscale score than those in normal BMI group (Males: $F_{(3,2276)}$= 5.11, p= 0.003; Females: $F_{(3,2563)}$= 6.74, p= 0.001). Additionally, males with obesity status also had higher score on DIF subscale than normal BMI males and overweight males by mean differences of 1.25 ±0.37 (p= 0.005) and 1.12 ±0.38 (p= 0.019), respectively.

Further analysis of this relationship, at 46 years, revealed that similar significant mean differences in the EOT ($F_{(3,4630)}$= 5.22, p= 0.001) and DIF ($F_{(3,4630)}$= 4.86, p= 0.002) subscale scores adjusted for sex, and SES. Bonferoni's post-hoc assessment showed that both obese males and females had higher mean EOT subscale scores than normal BMI males and females and the mean differences for those males and females were 0.94 ±0.37 (p< 0.001) and 0.89 ±0.21 (p< 0.001), respectively at age of 46 years. Males in obesity and overweight groups also had higher score on DIF subscale than males in normal BMI group by mean differences of 1.08 ±0.28 (p= 0.001) and 1.03 ±0.26 (p< 0.001), respectively. Additionally, females with obesity status had higher score on DIF subscale than females in normal BMI group by mean differences of 0.83 ±0.22 (p= 0.001).

Figure 3.2, shows a J-shaped/ U-shaped relationship between the mean TAS-20 and BMI quartiles at 16, 31 and 46 years of age. The significant associations are indicated on Figure 3.2. The BMI quartiles plot shows that heavier people in the general population scored higher on TAS-20, which indicates that they were inclined to be less able to identify and describe emotions, regardless of their age.

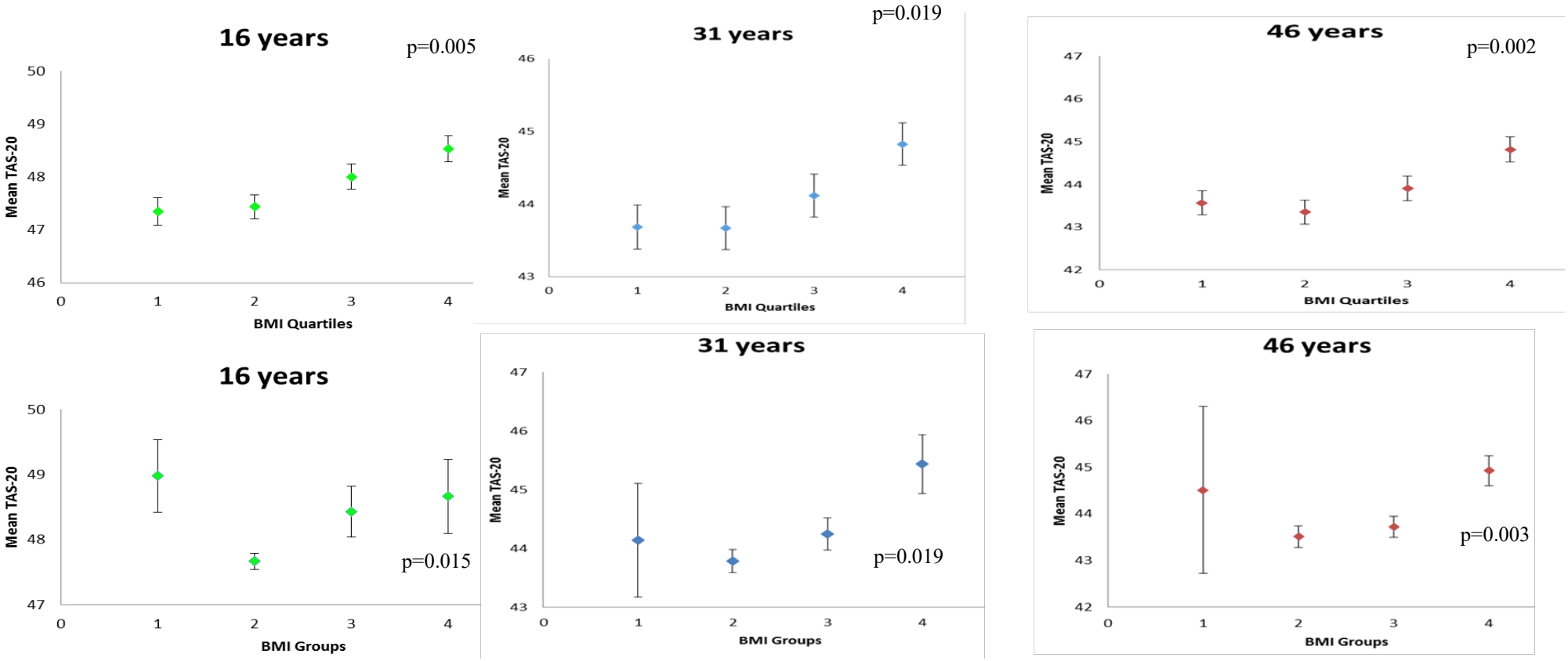

Figure 3.2 BMI groups according to WHO classifications and BMI quartiles were used as exposures to test on the mean total TAS-20 score plots above. The mean TAS-20 plots are by age of 16 years in the NFBC1986 (n=5698), 31 years (n=4841) and 46 years (n=5404) in the NFBC1966. Data presented are estimated marginal means (±SEM) derived from the model, adjusted for sex at 16 age of years in the NFBC1986. For the TAS-20 mean difference against BMI quartiles in the NFBC1966, ANCOVA models was adjusted for sex and SES. BMI was subdivided into quartiles at each timepoint measured for NFBC1966 and NFBC1986; $Q1_{31y}$: 15.32-21.75 kg/m^2, $Q2_{31y}$: 21.76-23.86 kg/m^2, $Q3_{31y}$: 23.87-26.50 kg/m^2, $Q4_{31y}$: 26.51-54.32 kg/m^2, $Q1_{46y}$: 16.06-23.57 kg/m^2, $Q2_{46y}$: 23.58-26.15 kg/m^2, $Q3_{46y}$: 26.16-29.41 kg/m^2 and $Q4_{46y}$: 29.42-73.81 kg/m^2. For the NFBC1986, BMI quartiles cut-off points were $Q1_{16y}$: 16.79-18.89 kg/m^2, $Q2_{16y}$: 18.90-20.44 kg/m^2, $Q3_{16y}$: 20.45-24.65 kg/m^2, $Q4_{16y}$: 24.66-39.87 kg/m^2. The adjusted-p value shown was a significant association between BMI (groups and quartile) and TAS-20 total score at respective age/timepoint.

3.3.5 Longitudinal analysis of alexithymia and obesity

Following the cross-sectional analyses at each time-point, a longitudinal analysis was carried out in all NFBC1966 participants who had data available at both 31 and 46 years of age (n=3274). A sex difference was observed in ΔBMI and ΔWHR (all $p<0.001$), but not in ΔTAS-20 score at both time-points. Females (n=1878) had higher mean ΔBMI than males (n=1396) by 0.65 (±0.11) ($t_{(3272)}=5.91$, $p< 0.001$). In contrast, males had higher mean ΔWHR than females by 0.01 (±0.002) ($t_{(3080)}=4.24$, $p< 0.001$).

Females and males, on average, gained 2.66 (±3.25) kg/m^2 and 2.02 (±2.56) kg/m^2 respectively and sex difference was seen in overall BMI comparison between both timepoints (31y: $p<0.001$, 46y: $p=0.02$) (Table 3.4). Over the 15-year study period, 63.8% of females (n=150) were in the obese category at both timepoints, as shown in Table 3.5, compared to of 36.2% males (n=85).

Our data indicate that the observed changes in TAS-20 score were related to changes in weight. Individuals with the smallest change in BMI (+/- 5% kg/m2) (n=888) had, on average, stable mean TAS-20 score over time (43.6±10.1 and 43.7±9.8, respectively). This was particularly evident for the EOT subscale. There were sex differences in the scores EOT subscale for all three BMI transition groups (remaining obese, transitioning to normal/underweight, or transitioning to overweight/obese) at both timepoints (all $p<0.001$) (Table 3.4). Despite these differences, both sexes exhibited similar relationships between weight change and the EOT subscale (Males: $r=0.17$; $p<0.001$, Females: $r=0.13$; $p<0.001$).

Individuals with lower scores on the EOT subscale lost more weight/gained less weight than those with higher scores on that subscale. Individuals who lost weight showed decreased EOT

scores (-1.15 score change on average) and those who gained weight had increased EOT scores (+2.38 score change) ($p<0.001$).

Table 3.5 Transition between body mass index groups in NFBC1966 participants over 15 year's time. Data is presented as mean (±SD) for continuous variables. BMI ranges were classified according to the WHO International Classification system. Numbers marked in bold indicate significant on the 95% confidence limit.

Obese	**31 years**		**Overall Mean**	**¥p-value**	**46 years**		**Overall**	**¥p-value**	***p-value**
	Males	**Females**			**Males**	**Females**	**Mean**		
N(%)	85(36.2)	150(63.8)	235		85(36.2)	150(63.8)	235		
TAS-20	47.5(±11.4)	44.3(±10.6)	45.5(±10.9)	**0.03**	49.2(±11.0)	44.5(±10.7)	46.2(±11.0)	**0.002**	0.213
DIF	13.6(±5.5)	14.4(±5.2)	14.1(±5.3)	0.25	14.8(±5.8)	14.6(±5.2)	14.6(±5.4)	0.71	0.131
DDF	12.0(±4.2)	10.8(±4.1)	11.2(±4.2)	**0.034**	12.2(±4.0)	10.6(±3.6)	11.2(±3.8)	**0.002**	0.971
EOT	21.9(±4.8)	19.1(±4.5)	20.1(±4.8)	**<0.001**	22.2(±3.9)	19.4(±4.3)	20.4(±4.4)	**<0.001**	0.327
Transitioned to normal or underweight	**Males**	**Females**	**Overall Mean**	**¥p-value**	**Males**	**Females**	**Overall Mean**	**¥p-value**	***p-value**
N(%)	38(50.7)	37(49.3)	75		38(50.7)	37(49.3)	75		
TAS-20	47.4(±9.7)	40.6(±7.7)	44.0(±9.4)	**0.001**	47.3(±8.7)	43.0(±11.1	45.2(±10.2)	0.065	0.19
DIF	13.6(±4.9)	13.1(±3.8)	13.3(±4.3)	0.60	14.0(±4.7)	13.7(±4.7)	13.9(±4.7)	0.748	0.271
DDF	11.4(±4.1)	9.7(±4.2)	10.6(±4.2)	0.07	11.6(±3.8)	11.3(±4.2)	11.4(±4.0)	0.805	**0.04**
EOT	22.3(±3.5)	17.8(±3.3)	20.1(±4.1)	**<0.001**	21.8(±3.7)	18.0(±4.3)	19.9(±4.4)	**<0.001**	0.661
Transitioned to overweight or obese	**Males**	**Females**	**Overall Mean**	**¥p-value**	**Males**	**Females**	**Overall Mean**	**¥p-value**	***p-value**
N(%)	342(39.4)	526(60.6)	868		342(39.4)	526(60.6)	868		
TAS-20	46.4(±9.7)	42.3(±10.2)	43.9(±10.2)	**<0.01**	46.7(±9.4)	42.4(±9.6)	44.1(±9.8)	**<0.01**	0.56
DIF	13.2(±4.4)	13.8(±4.8)	13.6(±4.7)	0.079	13.4(±4.5)	13.4(±4.6)	13.4(±4.6)	0.876	0.284
DDF	11.8(±4.0)	10.1(±3.7)	10.7(±3.9)	**<0.001**	11.7(±3.7)	10.2(±3.7)	10.8(±3.8)	**<0.01**	0.751
EOT	21.4(±4.6)	18.4(±4.4)	19.6(±4.7)	**<0.001**	21.5(±4.0)	18.8(±4.2)	20.0(±4.3)	**<0.001**	**0.029**

¥Sex comparisons at each time-point *Overall mean comparisons between time-points, 31-year vs 46-year.

At age of 46 years, there was a significant association between ΔBMI quartiles and mean TAS-20 scores at age of 46 years, adjusted for sex, marital status, education level and annual income ($F_{(8,2838)}$= 2.71, p= 0.04), as shown in Figure 3.3. The figure reveals a U-shaped trend found for both males and females in association between ΔBMI quartiles and the TAS-20 total scores at 46-year timepoint. Participants with the greatest ΔBMI (in a positive or negative direction) over the 15-year test period had higher mean TAS-20 score at age of 46 years.

Analysis by sex revealed some significant caveats to the statistical conclusion that alexithymia was associated with weight change. Significant mean difference on TAS-20 scores between ΔBMI quartiles was only observed in males ($F_{(3,1256)}$= 3.08, p= 0.027) not in females at age of 46 years.

Multiple linear regression models were conducted using the longitudinal set (n=3274) to predict BMI outcome on alexithymia measures. In the first model, alexithymia status at 31-years ($t_{(2972)}$= 2.27, β= 0.04, p= 0.02) was significantly predictive of BMI at the age of 46 years. Just over 1% of the total variance (adjusted R^2) of BMI was explained by alexithymia status (1.1% ±4.89), ($F_{(6, 2972)}$= 6.72, p< 0.001) in the overall best-fit model adjusted for sex and SES factors at 31-year (marital status, education level, employment, annual income).

In the second model, the TAS-20 total scores at 31 years were used as continuous independent variable to predict BMI at later age of 46 years. Sex, marital status, education level, employment, and annual income at age of 31 years were used as standard covariates. The TAS-20 total scores ($t_{(2972)}$= 2.24, β= 0.21, p= 0.03) and education level ($t_{(2972)}$= 4.04, β= -0.53, p< 0.001) at age of 31 years observed as significant predictors for BMI at 46 years. The total variance (adjusted R^2) of BMI at 46 years was explained by the TAS-20 total scores at 31 years (1.1% ±4.89), ($F_{(6, 2972)}$= 6.70, p< 0.001) in the overall best-fit model adjusted for sex and SES factors at 31-year (marital status, education level, employment, annual income).

To test associations between TAS-20 subscales and BMI outcome, separate regressions were performed for each TAS-20 subscale: DDF, DIF and EOT as an independent variable with sex and SES as standard covariate adjustment. This was to avoid violation of multi-collinearity assumptions in the regression models.

DIF ($t_{(2972)}$= 2.07, β= 0.04, p= 0.04) and EOT ($t_{(2972)}$= 2.19, β= 0.04, p= 0.03) subscales at 31-year were found to be significant predictors for BMI outcome at age of 46 years. The total variance (adjusted R^2) of BMI was explained by 1.1% (±4.89), (DIF: $F_{(6, 2972)}$= 6.58, p< 0.001, EOT: $F_{(6, 2972)}$= 6.66, p< 0.001). There was no evidence that DDF was associated with BMI in the multiple regression model.

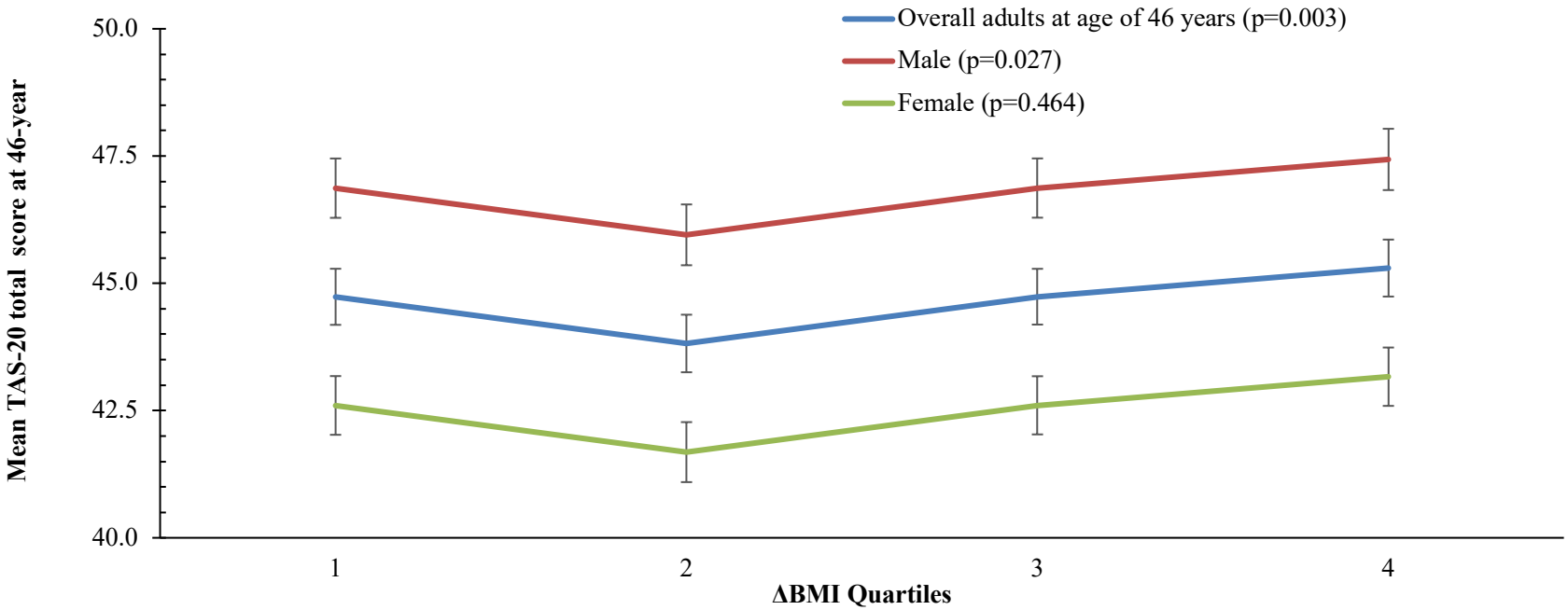

Figure 3.3 Longitudinal analysis between TAS-20 total scores and change of BMI quartiles at 46 year timepoint in the NFBC1966. Blue line represented estimated marginal means (±SEM) derived from the ANCOVA model with covariates adjustments (sex, marital status, employment, education level and annual income) for overall adults at age of 46 years. Red line represented estimated marginal means (±SEM) derived from the ANCOVA model with covariates adjustments (marital status, employment, education level and annual income) for males at age of 46 years. Green line represented estimated marginal means (±SEM) derived from the ANCOVA model with covariates adjustments (marital status, employment, education level and annual income) for females at age of 46 years.

3.4 DISCUSSION

In this chapter, the main aim was to ascertain the relationship between alexithymia and obesity related phenotypes in two European general population birth cohorts; NFBC1966 and NFBC1986.

As was hypothesised, there are associations between BMI and TAS-20 scores in general adult and adolescent populations. We observed that the TAS-20 total scores are associated with obesity measures (BMI and WHR) separately at 16 years (NFBC1986), 31 years and 46 years (NFBC1966). In addition, participants with alexithymia status (TAS-20 ≥61) had nominally higher mean BMI and WHR compared to non-alexithymic participants.

Exploration of the changes in BMI and TAS-20 over a period of 15 years showed that the relationship between BMI change and TAS-20 score is significant at the 46-year timepoint. The TAS-20 is a multi-faceted construct and from our dataset, we can observe a clear pattern of DIF and EOT subscales manifestation in both males and females on overall associations between BMI groups and TAS-20 differences at age of 31 and 46 years.

The concept of alexithymia being a stable personality trait/construct, as opposed to a state-dependent trait has been debated for years. This issue may stem from the concept of primary and secondary alexithymia introduced by Freyberger (1977) (114). Primary alexithymia is suggested to be a personality trait, while secondary alexithymia results from trauma and stress, or even health problems, during adolescence or adulthood (256). It has, for example, been argued that alexithymia may simply be a covariate of other health problems (113). Authors have found that alexithymia is a state-dependent construct attribute to depression, anxiety and emotional distress associated with trauma (26, 257, 258). However, it is also probable that alexithymia includes both state and trait components, due to its complex manifestation within

cognitive and affective processing style according to the generally accepted James Gross' models of emotion regulation (186, 187). Since depression is associated with obesity and alexithymia (259, 260), the next Chapter 4 moves on to investigate the relationship between alexithymia and depression and how it interacts with BMI status and weight change.

Despite no direct association between ΔTAS-20 and ΔBMI over the 15 years' time period of the longitudinal analysis, multiple regression models revealed that alexithymia status (TAS-20 ≥61) and the TAS-20 total score at age 31 can predict BMI at later age of 46 years. Interestingly, the TAS-20 total scores and its subscales (DIF: difficulty identifying feelings/emotions and EOT: externally-oriented thinking) at 31 years we also significant predictors for BMI at the later age. These results provide further support for the hypothesis that there is a relationship between BMI and alexithymia.

Several researchers have attempted to ascertain the direction of a causal relationship between obesity and emotional states (261-265), but with inconsistent findings, probably because of the complex nature of these phenotypic traits, and the different psychological constructs used across studies. Thus, several questions remain unanswered at present. Since obesity and eating disorders have been previously associated with alexithymia, our results further reinforce existing evidence that alexithymia impacts on energy balance in an unknown and, probably, complex manner.

There is a well-established relationship between socio-economic status and BMI (266-268), but correction for socioeconomic status did not substantially change the association results. Salminen *et al.* (1998) (269) suggested that an individual's ability to deal with emotions is affected by actual socioeconomic status. Our results are in line with previous Finnish adult and adolescence population studies which showed that age, male sex, marital status and low socio-economic status are associated with alexithymia (227, 270, 271). Additionally, as seen in

previous alexithymia studies in Finnish population (227, 270), the proportion of males (54% of the total population above the threshold) was marginally higher in alexithymia cases. At the age of 16 years, our demographics results showed that family type, parent's lower education level, having an unemployed father and lower annual income were associated with alexithymia. Children from a single parent family had higher TAS-20 scores, in accord with reports that lack of emotional and social support from family during childhood predict alexithymia in adulthood (272).

Additionally, we observed sex differences in alexithymia prevalence in both adult and adolescent study populations. In the adolescent NFBC1986 cohort, alexithymia prevalence was higher in females compared to males at age of 16 years, in contrast to the results from the adult NFBC1966, cohort, where males were more frequent among alexithymia cases, as reported in previous alexithymia studies in the Finnish population (190, 269, 273-276). Joukamaa *et al.* (2007) was first to study alexithymia in the Finnish adolescent population (using the same NFBC1986 dataset), and they have previously reported that the prevalence of alexithymia at 15–16-years was higher in females compared to males. These results corroborate the ideas of Lumley and Sielky (256) (2000), who suggested that alexithymia is more likely to be developed as a consequence of emotional trauma in the childhood for females. Our findings can be seen as possibly providing tentative support for this theory.

Alexithymia has been reported to be associated with obesity and with eating disorders as well as a number of other physical and psychological disorders (22, 25-27, 49, 61, 62, 192-194). The relationship between obesity, depression, psychosocial function and working ability has been explored in previous NFBC1966 investigations and we found the same weight trajectories as in Nevanperä *et al.* (2015) (277) reporting that the obesity prevalence rate doubled over a 15 year period. The analysis reported here is the first to consider TAS-20 score and BMI or WHR in this cohort and overall, internationally, the first longitudinal analyses on this subject.

Over a period of time, people generally become obese because of a complex interaction between psychological, biological and environmental factors that influence obesity mechanisms. Poor emotion regulation can influence health behaviour and lifestyle. Alexithymic individuals have been shown to have poorer nutritional intake (57) and decreased immune functioning (278).

3.5 Conclusion

The main goal of the current study was to explore the relationship between BMI and alexithymia in general adult and adolescent Finnish population cohorts. We observed that alexithymia has an influence in BMI/weight over time. Future studies regarding the association between changes in BMI/weight and emotion processing deficits are needed, for direction of causality to be fully determined. To date, there has been no information in the literature exploring the phenotypic and genetic profile of alexithymia and BMI simultaneously over the life course in general populations especially among adolescence.

CHAPTER 4 Depression and its Relationship with Alexithymia and Obesity in the Northern Finnish Adult Population

In the previous chapter, the results presented evidence a relationship between emotion processing deficits (as measured by TAS-20) and measures of obesity, both cross-sectionally in adolescence, adulthood and middle age, and over a 15-year time period in adulthood. One possible confounder or contributing factor in those analyses is depression status. Here, I investigate the relationship between TAS-20 score and depression in the NFBC1966, and the implications of this relationship for BMI.

Depression is a common mental health condition which has considerable impact on both obesity (279) and emotion processing (195). However, the direction of causality between depression and obesity is not yes understood. Although formal causal analyses e.g. using genetic instruments (Mendelian Randomisation) are outside the scope of the present study, I will explore observationally the role of depression in my study context.

Alexithymia has been previously reported to be associated with depression (189, 190) but there are inconsistencies in reports examining the relationship between severity of depression and TAS-20 score (and its subscales; DDF, DIF and EOT, in particular) (45). A review by Stunkard *et al.* (2003) (280), described how the complex nature of associations between depression and obesity was first established using a socio-psychological theoretical framework, designed by Baron and Kenny (1986) (281). The review highlighted the distinction between moderators (effect modifiers) and mediators in terms of the relationship between depression and obesity. The current methods for mediation analyses has been criticized and the use of genetic instruments to establish mediation have been suggested as a more robust method (282). Moderators are variables that directly affect the strength of association between the two conditions, whereas a mediator is a variable through which the independent variable influences the dependent variable (283). For example, Markowitz *et al.* (2008) proposed that female sex,

severity of obesity, and higher socioeconomic status (SES), were moderators for the obesity-depression relationship, since these factors increase the likelihood of obese individuals having depression (9).

There have been controversial debates over whether alexithymia is a stable trait (as a risk factor for depression) (284-286) or a state (as a defensive consequence of depression) (55, 189, 190). In addition, it has been suggested that there is an overlap, or a shared construct of emotional processing deficits between depression and alexithymia (191). Emotional inhibition strategies may be used as coping mechanisms by people with depression, leading to a higher prevalence rate of alexithymia among these patients, as compared to the general population (196).

Alexithymia has its own genetic components which are still largely unknown, whilst the known genetic components of depression do not explain the correlation with alexithymia (70). In our adult dataset, alexithymia is associated with male sex, single, lower education and income (refer Chapter 3). We also found that males and females in the adult NFBC1966 dataset exhibited similar relationships between weight change and the EOT subscale.

There have been inconsistent reports of sex-specific association between TAS-20 scores and the severity of depression (276, 287-290) . This inconsistency may be due to small sample sizes that could limit the power for sex-stratified association analyses. In addition to that, SES variables (i.e. sex, marital status, educational level, occupation status, income level) may have independent on the association between alexithymia and depression severity. Furthermore, differences in the measurement of obesity, depression, and alexithymia may also contribute to the conflicting outcomes of the various efforts to elucidate the relationships among them.

Since alexithymia may be co-morbid with depression, it is possible that the association of BMI with TAS-20 score, (as described in detail in Chapter 3) may be a reflection of, or through depression, rather than an independent effect. The formal mediation analyses are out of scope

of this thesis thus the work in this chapter was aimed at elucidation of the relationship between TAS-20 scores and depression, as well as the possible contribution of such a relationship to BMI, in this non-clinical general population.

4.1.1 Aims of the study

i. To investigate the relationship between TAS-20 scores and measures of depression.
ii. To determine the association between depression and obesity measures (BMI and WHR) and determine whether this is an independent of TAS-20 scores.
iii. To determine whether alexithymia or depression can predict BMI in the general population.

4.1.2 Null hypotheses

i. There is no association between alexithymia and depression among the adults in the Northern Finnish population.
ii. There is no association between depression and obesity measures (BMI and WHR), and alexithymia or depression, independently of each other, do not associate with obesity among the adults in the Northern Finnish population.
iii. Alexithymia or depression do not predict BMI in the general population.

4.2 METHODS

The study cohort involved in this chapter was the NFBC1966 cohort. The cohort characteristics and relevant methods were as described in Chapter 2. Depression was self-rated in two ways; 1) severity of current depressive symptoms by the 13-item depression subscale (HSCL-13) and 2) clinical depression diagnosis by a physician.

Firstly, the depressive symptoms score was generated by the sum of the 13-item depression subscale (HSCL-13) divided by the number of items answered from HSCL-25 questionnaire set. Current depressive symptoms were measured using a cut-off score of 1.75, which gave the same prevalence estimates as a diagnostic, clinical interview for depressive and anxiety disorders (230, 231). Secondly, self-reported clinical depression was defined by one item in the questionnaire: whether the study participant had ever been diagnosed as having depression by a physician (No/Yes). Both pieces of data were collected at 31-year and 46-year timepoints.

Association analyses between depression, alexithymia and obesity measures were performed using either Pearson's (r) correlation, analysis of covariance (ANCOVA) or multiple linear regression, as appropriate, to answer specific research questions. As standard, ANCOVA models performed at the cross-sectional level were adjusted for covariates (sex, marital status, educational level, occupation and annual income). Bonferroni adjustment (291) was used for multiple testing correction of main effects comparisons and interactions in ANCOVA models.

For longitudinal analysis, the change in the emotional constructs (HSCL-13 and TAS-20 scores) was calculated as ΔHSCL-13 = HSCL-13_{46y} – HSCL-13_{31y} and ΔTAS-20 = TAS-20_{46y} – TAS-20_{31y}. For all analyses, the Statistical Package for Social Sciences (SPSS for Windows, version 22.0, 2004, Chicago, IL, USA) was used.

4.3 RESULTS

In this section, I first describe depression demographics at 31-year and 46-year timepoints in the NFBC1966. The second subsection (4.3.2) examines the relationships between obesity, alexithymia and depression in the NFBC1966 at cross-sectional and longitudinal levels.

4.3.1 Depression demographics at 31-year and 46-year timepoints in NFBC1966

From the whole NFBC1966 cohort, 6810 and 5844 participants completed HSCL-13 and had available SES data, at 31-year and 46-year timepoints, respectively.

These participants were included in the demographic depression analysis. For history of clinical depression diagnosis, 4.3% (n=298) and 5.2% (n=304) of 31 and 46 years old participants, respectively had been diagnosed with depression by physician at some point in their lifetime. These people had a known depression diagnosis, and were experiencing significant symptoms at the time of data collection.

Table 4.1 presents the demographics of overall current depressive symptoms at the 31-year timepoint. Univariate analysis by sex shows that females who were single, divorced or widowed had higher HSCL-13 scores than married participants, by 0.07 (±0.02), 0.20 (±0.03), and 0.28 (±0.19), respectively at 31 years of age (all $p< 0.05$). Similar results were seen in males: divorced participants had higher HSCL-13 scores than participants who were married, by 0.35 (±0.04), and 0.32 (±0.04), respectively at 31 years of age (all $p< 0.001$).

Table 4.2 presents the overall demographics of current depressive symptoms at the 46-year timepoint. Participants who were divorced at 46 years of age had significantly higher mean HSCL-13 scores than those with other marital statuses; married, or cohabiting, by 0.10 (±0.02) ($p< 0.001$), and 0.09 (±0.02) ($p=0.002$), respectively. Further analysis revealed that 46-year old females who were cohabiting, single, and divorced had higher mean HSCL-13 scores than married female participants, by 0.07 (±0.02) ($p=0.01$), 0.09 (±0.02) ($p=0.004$), and 0.13 (±0.02) ($p <0.001$), respectively.

Only 1.9% (n=130; males=51, females=79) had both depressive symptoms and a diagnosis of clinical depression at the 31-year timepoint. Thus, these people had a known depression

diagnosis, and were experiencing significant symptoms at the time of data collection. At the age of 46 years, 5.2% (n=304; males=105, females=199) had both depressive symptoms and a previous diagnosis of clinical depression.

Table 4.1 Distribution of socio-demographic variables, by current depressive symptoms (HSCL-13) at the 31-year follow up.

Group	31-year Follow up N=6810	HSCL-13 <1.75 N= 5866	HSCL-13 ≥1.75 N=944	P-value
Male/Female (%)	3246/3564 (47.7/52.3)	2906/2960 (49.5/50.5)	340/604 (36.0/64.0)	**<0.0001**
BMI, kg/m^2 (SD)	24.52 (4.3)	24.5 (4.2)	25.5 (5.1)	0.737
WHR, cm (SD)	0.86 (0.1)	0.85 (0.1)	0.89 (0.1)	**0.002**
TAS-20 scores (SD)				
Total TAS-20	44.27 (10.31)	43.40 (9.90)	49.01 (11.21)	**<0.0001**
DIF	13.48 (4.83)	12.86 (4.40)	16.96 (5.64)	**<0.0001**
DDF	10.91 (4.83)	10.62 (3.78)	12.51 (4.11)	**<0.0001**
EOT	19.89 (4.65)	19.93 (4.64)	19.54 (4.76)	0.052
Marital status (%)				**<0.0001**
Married	3323 (49.1)	2964 (50.8)	359 (38.4)	
Cohabiting	1630 (24.1)	1431 (24.5)	199 (21.3)	
Single	1531 (22.6)	1244 (21.3)	287 (30.7)	
Divorced	283 (4.2)	193 (3.3)	90 (9.6)	
Widowed	4 (0.1)	3 (0.1)	1 (0.1)	
Education (%)				**<0.0001**
1 - low	23 (0.3)	16 (0.3)	7 (0.7)	
2	3440 (50.6)	2943 (50.2)	497 (52.6)	
3	2467 (36.3)	2112 (36.0)	355 (37.6)	
4 - high	873 (12.8)	788 (13.4)	85 (9.0)	
Income (%)				**<0.0001**
1 - low	1435 (23.3)	1153 (21.7)	282 (33.3)	
2	1607 (26.1)	1374 (25.9)	233 (27.5)	
3	1422 (23.1)	1265 (23.8)	157 (18.6)	
4 - high	1694 (27.5)	1520 (28.6)	174 (20.6)	
Occupation (%)				**<0.0001**
Employed	4743 (70.3)	4234 (72.8)	509 (54.4)	
Unemployed	1053 (15.6)	799 (13.7)	254 (27.1)	
Others	952 (14.1)	779 (13.4)	173 (18.5)	
BMI group (%)				**0.009**
Underweight	144 (2.1)	114 (1.9)	30 (3.2)	
Normal	4062 (59.8)	3513 (60.1)	549 (58.4)	
Overweight	2008 (29.6)	1744 (29.8)	264 (28.1)	
Obese	574 (8.5)	477 (8.2)	97 (10.3)	

Educational level categories: i) basic education or unfinished basic education, ii) completed 9-year basic education with or without vocational training or vocational school or post-secondary school, iii) completed 9-year basic education or matriculation examination with or without polytechnic education to iv) completed 9-year basic education or matriculation examination with university degree. 'Others' employment status: students, retired, and participants on paternity or maternity leave.

Table 4.2 Distribution of socio-demographic variables, by current depressive symptoms (HSCL-13) at the 46-year follow up.

Group	46-year Follow up N=5844	HSCL-13 <1.75 N= 4889	HSCL-13 ≥1.75 N=955	P-value
Male/Female (%)	2682/3162 (45.9/54.1)	2306/2583 (47.2/52.8)	376/579 (39.4/60.6)	**<0.0001**
BMI, kg/m^2 (SD)	26.84 (4.9)	26.68 (4.7)	27.65 (5.6)	**<0.0001**
WHR, cm (SD)	0.91 (0.08	0.91 (0.08)	0.92 (0.09)	0.283
TAS-20 scores (SD)				
Total TAS-20	43.99(10.0)	42.92 (9.4)	49.68 (11.2)	**<0.0001**
DIF	13.24 (4.6)	12.54 (4.1)	16.92 (5.3)	**<0.0001**
DDF	10.73 (3.7)	10.39 (3.6)	12.56 (4.2)	**<0.0001**
EOT	20.02 (4.4)	19.99 (4.4)	20.19 (4.4)	0.248
Marital status (%)				**<0.0001**
Married	3451 (59.3)	2997 (61.5)	454 (47.7)	
Cohabiting	1074 (18.5)	899 (18.5)	175 (18.4)	
Single	688 (11.8)	520 (10.7)	168 (17.7)	
Divorced	580 (10.0)	434 (8.9)	146 (15.4)	
Widowed	28 (0.5)	20 (0.4)	8 (0.8)	
Education (%)				**<0.0001**
1 - low	92 (1.6)	65 (1.3)	27 (2.8)	
2	1985 (34)	1602 (32.8)	383 (40.1)	
3	1287 (22.1)	1082 (22.2)	205 (21.5)	
4 - high	2470 (42.3)	2131 (43.7)	339 (35.5)	
Income (%)				**<0.0001**
1 - low	1166 (22.7)	868 (20.1)	298 (36.2)	
2	1338 (26.1)	1106 (25.7)	232 (28.2)	
3	1154 (22.5)	1010 (23.4)	144 (17.5)	
4 - high	1473 (28.7)	1324 (30.7)	149 (18.1)	
Occupation (%)				**<0.0001**
Employed	5006 (88.9)	4304 (91.2)	702 (76.6)	
Unemployed	319 (5.7)	219 (4.6)	100 (10.9)	
Others	306 (5.4)	192 (4.1)	114 (12.4)	
BMI group (%)				**<0.0001**
Underweight	40 (0.7)	30 (0.6)	10 (1.1)	
Normal	2245 (38.5)	1919 (39.3)	326 (34.2)	
Overweight	2310 (39.6)	1966 (40.3)	344 (36.1)	
Obese	1237 (21.2)	965 (19.8)	272 (28.6)	

Educational level categories: i) basic education or unfinished basic education, ii) completed 9-year basic education with or without vocational training or vocational school or post-secondary school, iii) completed 9-year basic education or matriculation examination with or without polytechnic education to iv) completed 9-year basic education or matriculation examination with university degree. 'Others' employment status: students, retired, and participants on paternity or maternity leave.

In the cross-sectional dataset, 13.9% (n=944) and 16.3% (n=955) passed the threshold for current depressive symptoms (HSCL-13≥1.75) at age of 31 years and 46 years, respectively. There is a statistically significant difference in the prevalence of depressive symptoms in the longitudinal dataset (n=5774) [31y: 13.2% (n=765). 46y: 16.2% (n=935), $\chi^2_{(5774)}$=409.8, p< 0.001]. Approximately 5.5% (n=316) remained depressed as measured by HSCL-13, but only 2.4% (n=137) reported having previous clinical depression diagnosis at both timepoints (longitudinal group).

Among those who reported a history of clinical depression diagnosis by physician at both timepoints, the percentage of females (66.7%, n=91) was higher than of males (33.3%, n=46). Females (67.4%, n=213) were also more commonly presenting current depressive symptoms (HSCL-13≥1.75) than males (32.6%, n=103). On the other hand, older males had a higher rate of current depressive symptoms than younger males ($t_{(2652)}$=6.14, p< 0.001) as shown in Table 4.2.

At each timepoint, a similar sex difference was seen: females had higher prevalence of current depressive symptoms (31y: 64%, n=604; 46y: 60.6%, n=579) compared to males (31y: 36%, n=340; 46y: 39.4%, n=376) (all p< 0.0001). There was no significant difference in the prevalence of current depressive symptoms between the two timepoints.

Married (n=67, 1.2%), and single (n=68, 1.2%) participants had similar rates of clinical depression diagnosis by physician, when compared at 31-year timepoint. Over the 15-year period, the prevalence of clinical depression increased (at 46-year timepoint: n=656, 11.4%). Married participants had the highest percentage of clinical depression diagnosis by physician (5.3%, n=307) compared to participants with other marital statuses: single (2.0%, n=116), cohabiting (1.9, n=108), divorced (2.1%, n=120) and widowed (0.1%, n=5) at 46-year timepoint ($\chi_{(4)}$= 95.43, p<0.0001).

Having basic education or unfinished basic education was associated with current depressive symptoms both at the age of 31 and at 46 years old (31y: $\chi_{(3)}$= 19.28, p<0.0001, 46y: $\chi_{(3)}$= 36.55, p<0.0001). Additionally, having basic education or unfinished basic education was also associated with history of clinical depression diagnosis ($\chi_{(3)}$= 12.21, p=0.007) at the 31-year timepoint. Being unemployed was associated with depressive symptoms, at both timepoints (31y: $\chi_{(2)}$= 84.36, p<0.0001, 46y: $\chi_{(2)}$= 300.97, p<0.0001) (unadjusted analyses).

Additionally, the lowest income level was also associated with history of clinical depression diagnosis (31y: $\chi_{(3)}$= 80.53, p<0.0001, 46y: $\chi_{(3)}$= 137.65, p<0.0001). There is a clear inverse relationship at both timepoints between the prevalence of clinical depression and the level of household income. As the income level increased, the depression rate decreased in the sample population.

4.3.2 Relationship between obesity, alexithymia and depression in NFBC1966

In this subsection, the effect of alexithymia and depression on BMI or BMI groups (WHO classifications) was investigated using ANCOVA and multiple linear regression models. The relationships between BMI groups and HSCL-13 scores, adjusted for alexithymia status and standard covariates, at 31 and 46 years old are shown in Figures 4.7 and 4.8 respectively. Overall, quadratic (non-linear) associations between BMI groups and HSCL-13 scores were present at each timepoint (31y: $F_{(3,3386)}$= 2.98, p= 0.03, 46y: $F_{(3,4144)}$= 4.82, p= 0.002), adjusted for alexithymia status, and standard covariates (sex, marital status, educational level, occupation and annual income) in an ANCOVA model.

Figure 4.1 below shows that, as previously mentioned, females had significantly higher HSCL-13 scores than males at 31 years old [mean difference= 0.11 (±0.05), p= 0.02] in normal, overweight and obese groups. In addition, there was a higher prevalence of clinical depression

diagnosis in females than in males, particularly in the underweight and obese groups at 46 years old ($\chi(3)= 24.11$, $p<0.0001$). In an ANCOVA model, we also observed that, among females, clinical depression at the age of 31 years had a significant effect on BMI at the 31-year timepoint ($F(1,1838)=4.77$, $p= 0.03$).

Figure 4.2 shows the significant associations between BMI groups and HSCL-13 scores at the 46-year timepoint ($F_{(3,4149)}= 7.68$, $p< 0.0001$) There was a significant 3-way interaction between sex, alexithymia and BMI group on the mean HSCL-13 difference at age of 46 years ($F_{(6,4144)}= 2.95$, $p= 0.007$), but not at the age of 31 years. Further analysis by sex revealed a significant 2-way interaction between alexithymia and BMI groups on the mean HSCL-13 difference among males in the NFBC1966 (31y: $F_{(3,1546)}= 4.67$, $p= 0.003$, 46y: $F_{(3,1849)}= 3.07$, $p= 0.027$). The alexithymia and BMI group interaction on HSCL-13 score was not seen among females at either timepoint.

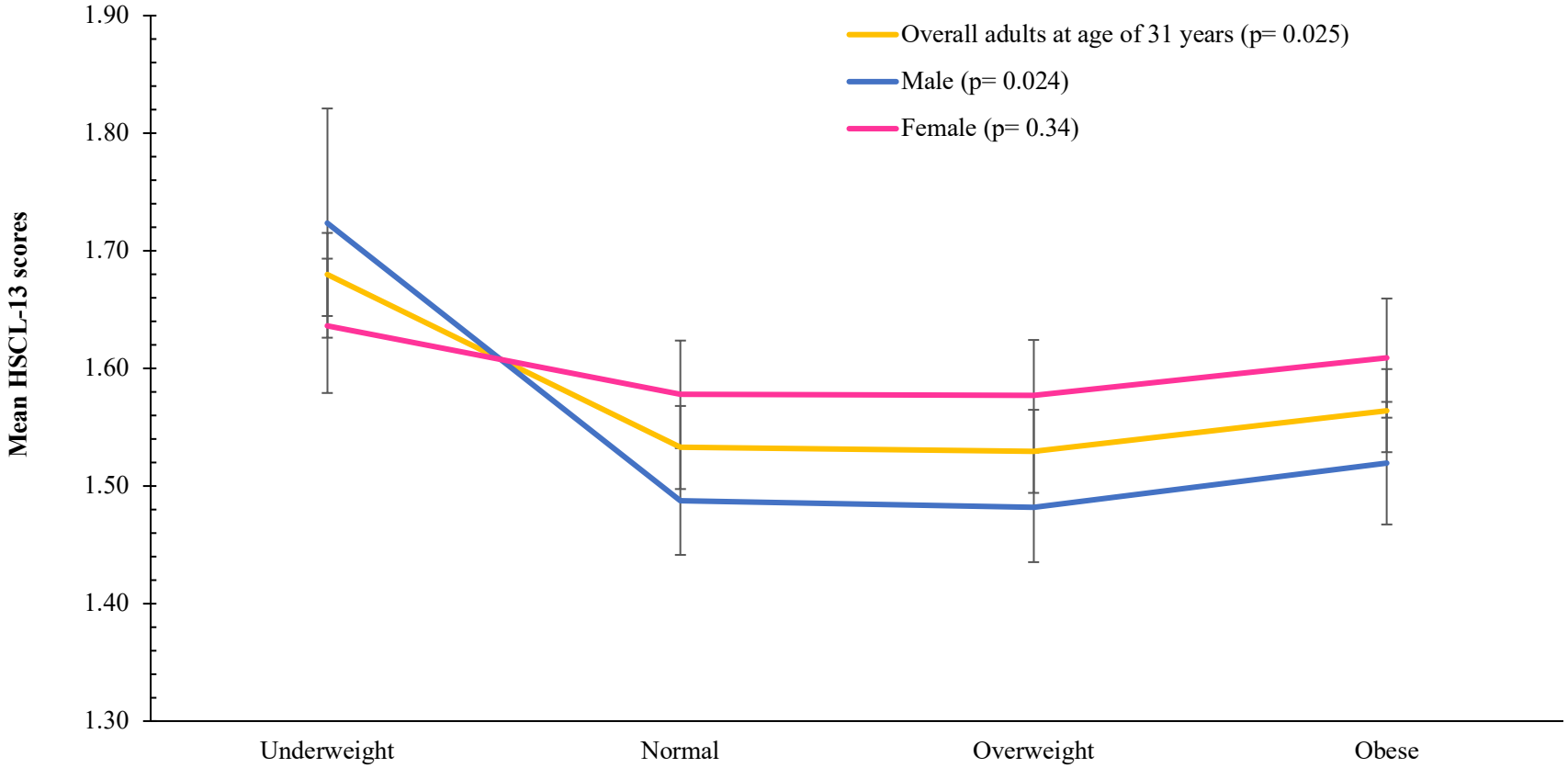

Figure 4.1 The mean plot between HSCL-13 scores and BMI classifications by overall and sex among 31-year adults in NFBC1966. Overall means were adjusted for alexithymia status and standard covariates (sex, marital status, educational level, occupation and annual income) in an ANCOVA model. Data presented derived from the ANCOVA models, covariates adjusted for Bonferroni's multiple testing corrections. The P value shown was a significance value between mean HSCL-13 score and BMI groups in overall adults, male and females in the NFBC1966.

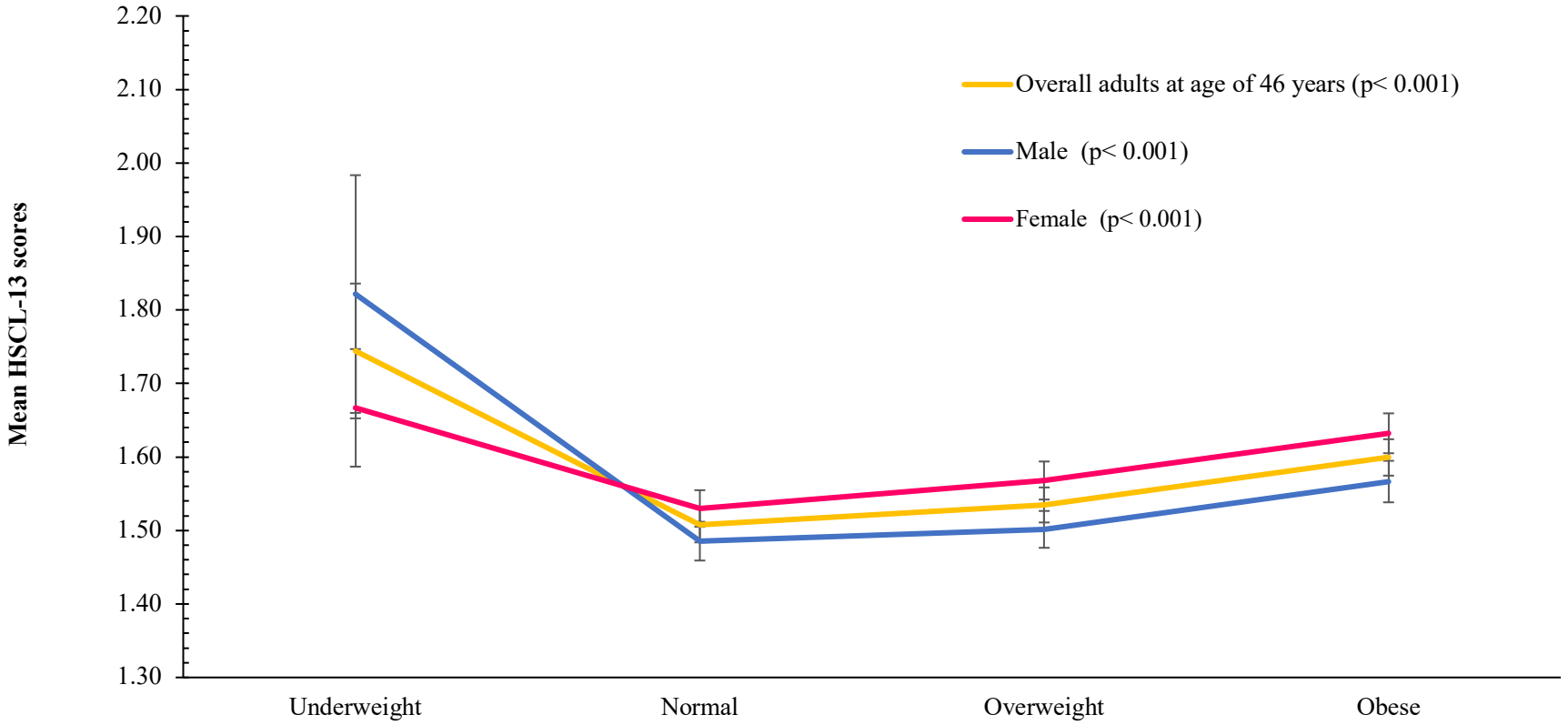

Figure 4.2 The mean plot between HSCL-13 scores and BMI classifications overall, and by sex among 46-year adults in NFBC1966. Overall means were adjusted for alexithymia status and standard covariates (sex, marital status, educational level, occupation and annual income) in an ANCOVA model. Data presented are derived from the ANCOVA models, with Bonferroni's multiple testing correction. The P value shown was a significance value between mean HSCL-13 score and BMI groups in overall adults, male and females in the NFBC1966.

4.3.2.1 Analysis by sex in relation to associations between HSCL-13 score and BMI groups

Males with alexithymia at the age of 31 (TAS-20≥61) had higher mean HSCL-13 score in all BMI groups: by 1.47±0.21, 1.51±0.04, 1.46±0.04, and 1.81±0.08 in underweight, normal, overweight, and obese groups, respectively, as compared to males without alexithymia (TAS-20≤60) at the age of 31 ($F_{(3,1546)}$= 4.67, p= 0.003). Indeed, among males, there were significant differences in mean HSCL-13 score between BMI groups at each timepoint (31y: $F_{(3,1546)}$= 3.70, p= 0.011, 46y: $F_{(3,1849)}$= 4.87, p= 0.002). In addition, there was a significant association between BMI, alexithymia and presence of current depressive symptoms at the 46-year timepoint (alexithymia: $F_{(1,2028)}$= 7.69, p= 0.006, and HSCL-13: $F_{(1,2028)}$= 6.73, p=0.01). There was significant interaction between sex and alexithymia status in association with BMI at the age of 46 years ($F_{(1,4149)}$= 4.13, p= 0.04).

At the 31-year timepoint, underweight males had higher mean HSCL-13 score than other BMI groups; normal, overweight and obese by 0.27±0.08 (p= 0.008), 0.28±0.08 (p= 0.004) and 0.24±0.09 (p= 0.04), respectively (all p< 0.05). In contrast, at the age of 46 years, males with obesity had higher mean HSCL-13 score than normal weight and overweight males, by (0.12±0.02, p= 0.009), and (0.12±0.02, p= 0.007), respectively. A similar trend was seen among females at age 46: participants with obesity had higher mean HSCL-13 score differences than females who were normal, or overweight, by (0.09±0.02, p< 0.0001), and (0.07±0.02, p= 0.001), respectively.

4.3.2.2 Inter-correlations between HSCL-13 scores, TAS-20 total scores (and subscales), and adiposity measures

Table 4.3 shows inter-correlations between HSCL-13 scores, TAS-20 total scores (and subscales; DDF, DIF, and EOT), and obesity measures (BMI and WHR) in the longitudinal dataset (n=3013). As mentioned earlier, the DIF subscale showed a relatively greater strength of correlation with HSCL-13 at both timepoints. A similar correlation value was found between the DIF subscale and HSCL-13 (31y: $r_{(3013)}$= 0.41, p<0.0001, 46y: $r_{(3013)}$= 0.43, p<0.0001) in the longitudinal dataset. Additionally, there are negative correlations between HSCL-13 at the age of 31 years and WHR at both ages (31y: $r_{(3013)}$= -0.09, p<0.0001, 46y: $r_{(3013)}$= -0.06, p<0.0001) (Table 4.3). Significant main effects of BMI groups on the HSCL-13 mean change (ΔHSCL-13) were seen at 31-year ($F_{(3, 3392)}$=5.27, p= 0.001) and 46-year ($F_{(3, 4153)}$=4.46, p= 0.004) timepoints (Figure 4.3 and Figure 4.4). The covariates; sex ($F_{(1, 3392)}$=5.77, p= 0.02) and marital status ($F_{(4,3392)}$=8.18, p< 0.01) were significantly associated with mean ΔHSCL-13.

Table 4.3 Overall correlation matrix between selected variables at longitudinal level[c]. Statistically significant medium effect of Pearson's correlation value (r: 0.30-0.49) between depressive symptoms (HSCL-13 scores), and TAS-20 total score and its subscale; difficulty of identifying feelings (DIF) are shown in bold. Obesity measures (BMI and WHR) are not adjusted by sex in this table. Numbers 1-14 of the Pearson correlation matrix represent variables as shown in the table's first column.

Variables	Mean (SD)	Pearson Correlations													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. HSCL-13 31Y	**1.34 (0.35)**	1	0.45*	0.25*	0.17*	0.24*	0.17*	**0.41***	0.29*	-0.07*	-0.06*	-0.02	0.03	-0.09*	-0.06*
2. HSCL-13 46Y	**1.37 (0.40)**	0.45*	1	0.22*	**0.31***	0.20*	0.29*	**0.31***	**0.43***	-0.01	0.01	0.03	0.09*	-0.02	0.02
3. TAS-20 31Y	44.0 (10.03)	0.25*	0.22*	1	0.64*	0.82*	0.54*	0.78*	0.50*	0.70*	0.46*	0.09*	0.09*	0.17*	0.21*
4. TAS-20 46Y	**44.08 (9.85)**	0.17*	**0.31***	0.64*	1	0.54*	0.85*	0.47*	0.81*	0.46*	0.70*	0.11*	0.10*	0.18*	0.22*
5. DDF 31Y	10.84 (3.85)	0.24*	0.20*	0.82*	0.54*	1	0.58*	0.57*	0.41*	0.37*	0.30*	0.05*	0.05*	0.14*	0.17*
6. DDF 46Y	10.78 (3.72)	0.17*	0.29*	0.54*	0.85*	0.58*	1	0.38*	0.64*	0.32*	0.39*	0.07*	0.05*	0.15*	0.18*
7. DIF 31Y	**13.42 (4.65)**	**0.41***	**0.31***	0.78*	0.47*	0.57*	0.38*	1	0.55*	0.22*	0.16*	0.03	0.07*	-0.01	0.02
8. DIF 46Y	**13.20 (4.58)**	0.29*	**0.43***	0.50*	0.81*	0.41*	0.64*	0.55*	1	0.19*	0.24*	0.09*	0.10*	0.06*	0.08*
9. EOT 31Y	19.73 (4.60)	-0.07*	-0.01	0.70*	0.46*	0.37*	0.32*	0.22*	0.19*	1	0.59*	0.12*	0.09*	0.27*	0.29*
10. EOT 46Y	20.10 (4.30)	-0.06*	0.01	0.46*	0.70*	0.30*	0.39*	0.16*	0.24*	0.59*	1	0.09*	0.08*	0.23*	0.27*
11. BMI 31Y	24.42 (4.07)	-0.02	0.03	0.09*	0.11*	0.05*	0.07*	0.03	0.09*	0.12*	0.09*	1	0.79*	0.50*	0.38*
12. BMI 46Y	26.79 (4.81)	0.03	0.09*	0.09*	0.10*	0.05*	0.05*	0.07*	0.10*	0.09*	0.08*	0.79*	1	0.36*	0.44*
13. WHR 31Y	0.85 (0.09)	-0.09*	-0.02	0.17*	0.18*	0.14*	0.15*	-0.01	0.06*	0.27*	0.23*	0.50*	0.36*	1	0.68*
14. WHR 46y	0.91 (0.08)	-0.06*	0.02	0.21*	0.22*	0.17*	0.18*	0.02	0.08*	0.29*	0.27*	0.38*	0.44*	0.68*	1

[c]Listwise N=3013

*Correlation is significant at the 0.01 level (2-tailed).

4.3.3 The relationship between BMI and HSCL-13 over the 15-year period

Figure 4.3 and Figure 4.4 depict the relationship between BMI group (WHO classifications) and ΔHSCL-13 at 31-year and 46-year timepoints, respectively. ΔHSCL-13 is calculated by ΔHSCL-13= $HSCL\text{-}13_{46y}$ – $HSCL\text{-}13_{31y}$ and is a dependent/constant variable at the age of 31 or 46 years (N.B. Figure 4.3 shows a negative direction of ΔHSCL-13 means because BMI groups at the age of 31 years were used as independent variable for the analysis).

Figure 4.5 shows the longitudinal relationship, stratified by alexithymia and depression status, between BMI group at the age of 31 years and depressive symptoms at later age, 46-year timepoint. There were significant interactions between BMI group, alexithymia status and sex on HSCL-13 score mean difference at age of 31 ($F_{(3,3390)}$= 3.91, p= 0.008) and 46 ($F_{(5,4152)}$= 2.51, p= 0.028) years. There were significant interactions between BMI group, alexithymia status and sex on HSCL-13 score mean difference at age of 31 ($F_{(3,3390)}$= 3.91, p= 0.008) and 46 ($F_{(5,4152)}$= 2.51, p= 0.028) years. Obese males and females who were alexithymic also passed the clinical cut off for depressive symptoms, HSCL-13≥ 1.75 at age 46 years.

To further analyse the interaction term between BMI group, alexithymia status and sex on HSCL-13 score, I constructed a regression modelling to predict future depressive symptoms at later age. A multiple linear regression analysis was conducted, adjusted for sex, education level, annual income level, marital status and type of employment. During the first step of the procedure, sex and SES factors; education level, annual income level, marital status and type of employment were entered into the model. Significant associations were seen between those covariates and HSCL-13 score at age of 46 years (all p<0.001).

In the second step, BMI groups, and alexithymia status at 31-years were entered, revealing in a significant association with HSCL-13 score at age of 46 years ($F_{(7, 2895)}$= 21.01, p< 0.0001).

The total variance of HSCL-13 scores explained by sex and alexithymia was 4.8% (±0.39), ($F_{(7, 2895)}$= 21.01, p< 0.0001). Male sex and alexithymia status (TAS-20 ≥61) at the age of 31 are significant risks factor for current depressive symptoms at the age of 46.

Obese participants had higher mean ΔHSCL-13 score than underweight, and normal-weight individuals, by 0.19 (±0.05, p= 0.002) and 0.07 (±0.03, p= 0.04), respectively at the 31-year timepoint. At the 46-year timepoint, there was a significant effect of BMI status ($F_{(3, 4153)}$=4.46, p= 0.004) on mean ΔHSCL-13, after controlling for the effect of alexithymia status ($F_{(1, 4153)}$=68.97, p< 0.001) and standard covariates. The covariates; sex ($F_{(1, 4153)}$= 8.65, p= 0.003), and annual income level ($F_{(1, 4153)}$=5.27, p= 0.001), were significantly related to the mean ΔHSCL-13 at age of 46 years (Figure 4.4). Males also had slightly higher mean ΔHSCL-13, by 0.04 (±0.01) (p=0.003) compared to females at the 46-year timepoint.

Participants who were obese at the age of 46 had higher mean ΔHSCL-13, by 0.06 (±0.02), 0.19 than normal-weight individuals (p= 0.003). Bonferroni's post-hoc analysis also revealed that participants with unemployed and 'other' employment status (students, retired, and participants on paternity or maternity leave), at the 46-year timepoint, had higher mean ΔHSCL-13, by 0.15 (±0.03), and 0.14 (±0.03), respectively than employed individuals (p< 0.001).

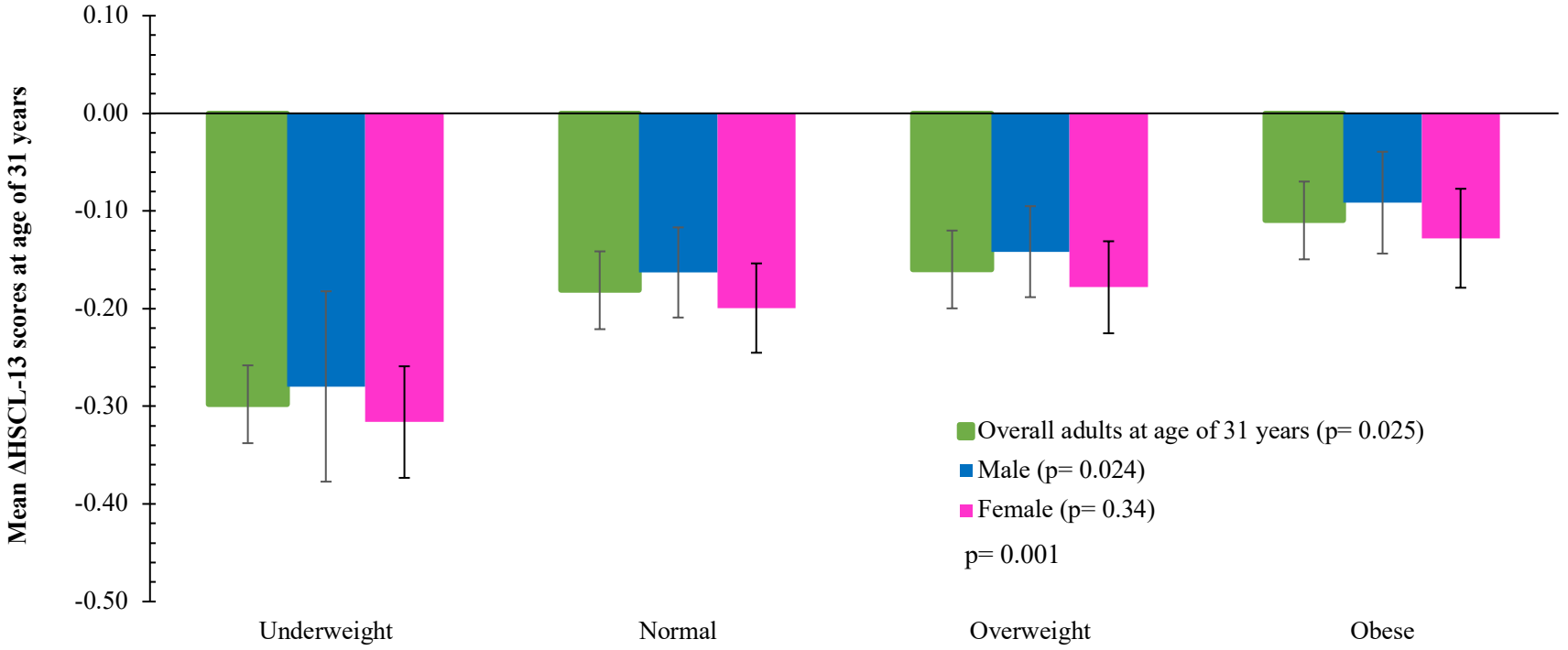

Figure 4.3 The mean ΔHSCL-13 plot by overall 31-year adults and by sex in the NFBC1966. Data presented are covariate-adjusted means for all groups with standard error of the means (SEM) derived from the ANCOVA models, adjusted for Bonferroni's multiple testing corrections. P value shown was a significant interaction between the effects of BMI groups on adjusted mean ΔHSCL-13 scores at age of 31 years

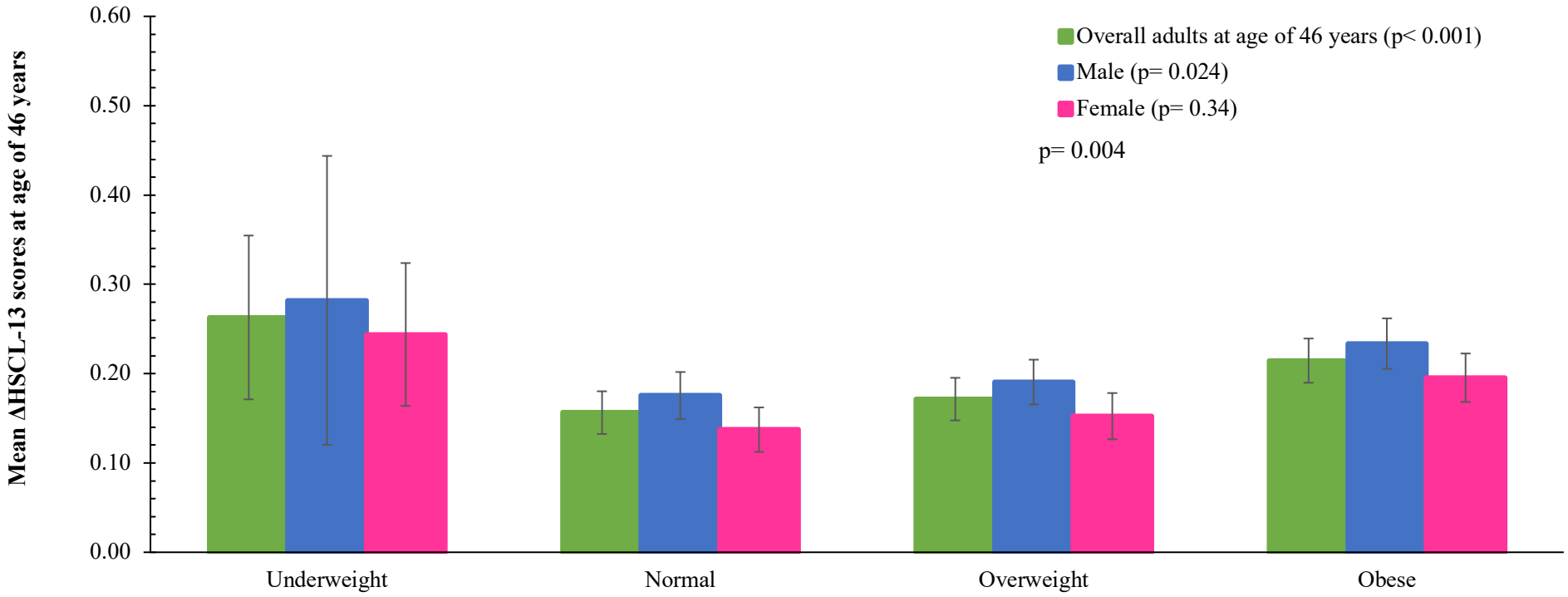

Figure 4.4 The mean ΔHSCL-13 plot by overall 46-year adults and by sex in the NFBC1966. Data presented are covariate-adjusted means for all groups with standard error of the means (SEM) derived from the ANCOVA models, adjusted for Bonferroni's multiple testing corrections. P value shown was a significant interaction between the effects of BMI groups on adjusted mean ΔHSCL-13 scores at age of 46 years.

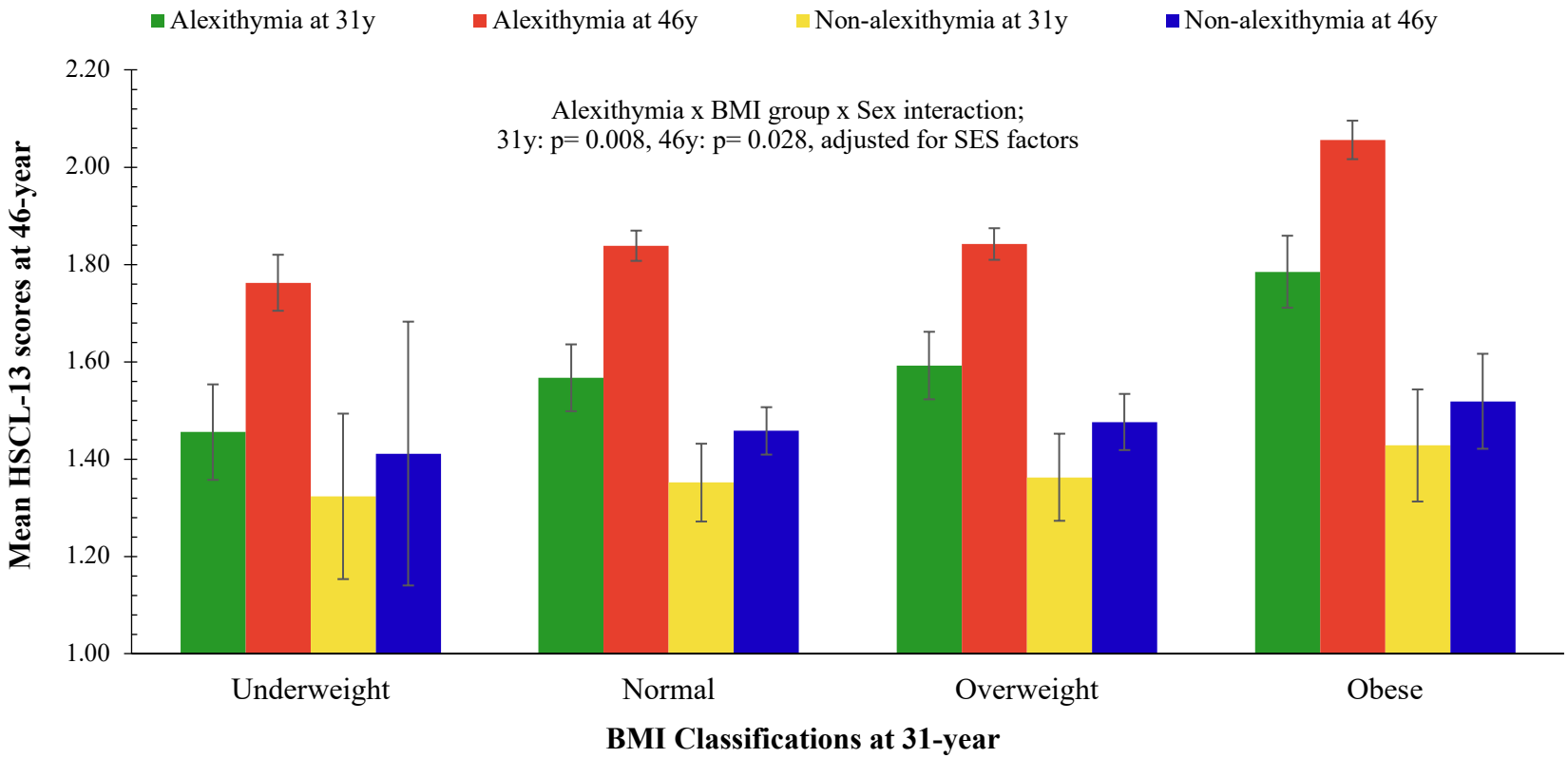

Figure 4.5 The longitudinal relationship between mean HSCL-13 scores and BMI group (WHO classifications) among general population adults in NFBC1966. Each bar plot represents alexithymia status (alexithymic vs non-alexithymic) at the ages of 31 and 46 years. Data presented are covariate-adjusted means for all groups with standard error of the means (SEM) derived from the ANCOVA models. P-value shown was for significant interaction between the effects of alexithymia status, BMI groups and sex on adjusted HSCL-13 scores at either age of 31 or 46 years

4.3.4 Can alexithymia and depression at age 31 predict future BMI?

A stepwise multiple linear regression was conducted to predict BMI at the later age (46-year), based on effects of TAS-20 score and HSCL-13 score (independent variables), adjusted for standard covariates (sex, marital status, educational level, occupation status, and income level) at age of 31 years. BMI at age 31 years was not included as covariate adjustment to avoid violating multi-colinearity assumption in a multiple linear regression model.

During the first step of the procedure, sex and SES factors; education level, annual income level, marital status and type of employment were entered into the model. Education level ($t_{(3403)}$= -4.74, β=-0.60, p< 0.001) stayed as a significant predictor for BMI at later age ($F_{(7,3402)}$= 7.52, p< 0.0001).

In the second step of the BMI prediction model, TAS-20 score and HSCL-13 score were entered, revealing only TAS-20 score ($t_{(3403)}$= 2.76, β=0.02, p= 0.006), and education level ($t_{(3403)}$= -4.06, β=-0.52, p< 0.001) as significant predictors of BMI difference at the age of 46 years ($F_{(7,3402)}$= 6.73, p< 0.0001). The total variance of BMI at the age of 46 years that was explained by the TAS-20 score and education level was 1.2% (±4.87), ($F_{(7,3402)}$= 6.73, p< 0.0001). Among adults in the NFBC1966, the TAS-20 (and not HSCL-13 score) and lower education level are predictors of BMI over 15 years.

4.4 DISCUSSION

The present study was designed to explore the relationship between two psychological constructs (alexithymia and depression) and their associations with BMI in the NFBC1966 cohort. The effect of alexithymia and BMI on depression was also studied at cross-sectional and longitudinal level.

In our dataset, alexithymia and depression are highly associated and there was an interaction between sex, alexithymia and BMI group in current depressive symptoms (measured by HSCL-13) at cross-sectional and longitudinal level. TAS-20 score is predictive of BMI (rather than HSCL-13 score) and lower education level predisposes to higher BMI over 15 years period. Male sex and alexithymia status at the age of 31 (TAS-20≥ 61) are significant risk factors for current depressive symptoms at later age.

Honkalampi *et al.* (2000) report that depression has a strong relationship with alexithymia in a general population(190). Our study further confirms that alexithymia is associated with current depressive symptoms among adults in the general Finnish population. The difficulty in identifying feelings (or emotions); DIF subscale, in particular, showed the strongest correlation with HSCL-13, compared to the total TAS-20 score and DDF subscales at both cross-sectional and longitudinal level. A meta-analysis from Li *et al.* (2015) confirmed that the total TAS-20 scores, DIF and DDF subscale are related to depression (292), but not the EOT subscale, due to rumination (persistent and recyclic negative thinking) that presents among depressed patients (293, 294).

The TAS-20 is a multi-faceted construct encompassing deficits in emotional regulation and social-cognitive functioning. The DIF subscale is associated with somatization and cognitive style (295) (296). We found that the DIF subscale is associated with current depressive symptoms, as measured by HSCL-13 scores.

Males with either divorced or unemployed status had higher HSCL-13 scores compared to females in the same categories, at the age of 46 years. Males also have a higher tendency to be alexithymic (55, 191, 256, 259, 271, 273) and our results support the suggestion that certain males might be predisposed to depressive symptoms, due to difficulty in identifying (and describing) feelings. However, a higher prevalence rate of current depressive symptoms among females was also observed in our dataset, as previously reported (297). A previous epidemiological study has suggested female predominance, from puberty to adult life in the prevalence, and incidence rate of depression and suggested that SES is one factor in this (298). However, there were discrepancies in reports of the extent to which socioeconomic factors associate with depression or obesity in the general population. In four large epidemiological studies from the United States, individuals with lower SES exhibited higher risk of developing depression and obesity (299) (see review by Everson *et al.* 2002). In contrast, the obesity prevalence rate has increased over the last four decades (1970s to 2000) in the high education group (college or higher) compared to the low and medium education groups among adults in the U.S [the first, second and third National Health and Nutrition Examination Surveys (NHANES I-III) (300)].

Results of previous meta-analyses and systematic reviews suggest that the causal link between obesity and depression was bi-directional in adults and adolescents (9, 301-307). I found that alexithymia and depression have a significant impact on obesity among adults in general Northern Finnish population. Faith *et al.* (2002) suggested there may be shared common genetic and environmental factors responsible for significant associations between obesity and depression in general population (308). Furthermore, individual differences in metabolic regulations and genetic variation may play roles that link obesity to depression, or *vice versa*. Additionally, significant sex differences and interactions were observed between BMI, alexithymia and depression in the NFBC1966 cohort.

A previous longitudinal study of the NFBC1966 cohort, using data from the 14-year and 31-year follow ups (by Herva *et al.* 2006) showed that adolescent obesity was associated with depressive symptoms in the later teenage years (152). Our results revealed sex differences in the interactions between obesity, depressive symptoms and alexithymia at later timepoints in the same Northern Finnish population cohort. Males with alexithymia and depression status had higher BMI than those individuals who were not alexithymic and depressed. In addition, males with either current depressive symptoms, or a history of clinical depression, had higher total TAS-20 score than females in the same depression groups, both at the age of 31 and at 46 years.

As a result of the findings presented here, sex interaction terms were included in the statistical analysis framework for a severely obesity clinical cohort (Chapter 6) and in the genetic epidemiological analysis in Chapter 5 of this thesis. The clinical implications of these findings are further discussed in Chapter 7.

CHAPTER 5 Genetic Association Analysis of Alexithymia and Depressive Symptoms in the Northern Finnish Adolescent and Adult Populations

5.1 INTRODUCTION

This chapter describes a genome-wide association study (GWAS) of TAS-20 total score carried out in a separate adult and adolescent Northern Finnish general population cohorts; 31-year olds adults (NFBC1966) and 16-year adolescents (NFBC1986). A GWAS of HSCL-13 score was also carried out separately in 31-year olds adults (NFBC1966). Any effect of interaction between sex and genetic factors, on the TAS-20 and HSCL-13 scores, was also investigated. These GWAS analyses are statistically underpowered but were conducted for training purposes and to provide analyses of single datasets for a larger consortium working on genetics of alexithymia.

Over the last decades, massive scale genomic research has been conducted with the motivation of understanding the genetic determinants (and, through that, the biology) of human diseases and phenotypic traits. Genome-wide association studies have been used as the standard approach for dissecting the genetic architecture of complex phenotypic traits, such as obesity, as reviewed by den Hoed *et al.* 2013 (309).

The genetic composition of alexithymia is largely unknown, despite the fact that there have been a few candidate gene-based studies conducted (82, 84, 85, 310). To date, there is only one exome functional variant genotyping analysis was conducted to study alexithymia using a case-control design in a college population setting (311).

As shown in the previous chapter, TAS-20 subscales (DIF and DDF) were moderately associated with current depressive symptoms, measured by HSCL-13 score, at the age of 31 and 46 years.

Extensive previous literature suggests that depression is genetically influenced (312-314), but the underlying biological mechanisms remain elusive. According to Picardi *et al.* 2011, moderate genetic correlation (0.65) was seen between alexithymia and depression, which suggests a common genetic basis between the two psychological constructs in general populations (70). Moreover, they also found substantial genetic correlation between TAS-20 subscales (DIF and DDF subscale) and depression.

It is of interest to understand the direction of causality in this relationship, but unfortunately the types of study undertaken are not ideal to elucidate this (116). In addition to our lack of understanding of the genetic basis of alexithymia, the genetic components contributing to depression in clinical and population studies remain incompletely understood. However, depression is known to be polygenic and multifactorial (119).

A standard approach to exploring causality relationships is bidirectional Mendelian Randomisation, in which genetic markers associated with a trait (derived from GWAS) are used as instrumental variables. GWAS data are already available for depression generally: here I carry out GWAS analysis of TAS-20 and of HSCL-13 scores, which would be the first step in this process. More advanced causal analyses are outside the scope of the present study.

Since I have access to individual-level phenotype and genotype data of an unselected, highly homogenous population of Northern Finland, I carried out GWAS separately on TAS-20 and HSCL-13 scores (as continuous traits) among 31-year old adults in NFBC1966. Since age and sex play a role in the relationship between alexithymia and depression (as described in Chapter 4), GWAS on TAS-20 total score was also conducted in NFBC1986 to identify genetic factors influencing TAS-20 score from age 15 through age 16, in adolescence. The investigation was also extended to identify any effect of interaction between sex and genetic factors, on the psychological traits under study.

5.1.1 Aims of the study

The main objectives of this study were:

i. To carry out GWAS for TAS-20 and HSCL-13 scores.

ii. To investigate possible genotype and sex-related effects on TAS-20 score and depressive symptoms in NFBC1966.

This study aimed to address the following research questions:

i. What genetic factors contribute to emotion processing deficits as measured by the TAS-20 scale?

ii. Is there any interaction between genetics and sex, for alexithymia and depressive symptoms?

5.1.2 Null hypotheses

i. There is no association between common genetic variants and TAS-20 and depressive symptom scores in the study populations.

ii. There are no genetic and sex interactions for TAS-20 and depressive symptoms scores among adult Finns.

5.2 METHODS

5.2.1 Study population

NFBC1966 and NFBC1986 participants who attended the 31-years' and 16-years' clinical examinations had blood samples drawn after overnight fasting. DNA was extracted for genotyping (NFBC1966: n=5753, NFBC1986: n=6266). Genotyping was performed using the Illumina HumanExome chip (which includes GWA content, along with 250,000 exonic

markers) for NFBC1966 by the Broad Institute Biological Sample Repository, United States: details of sample preparation and genotyping procedures can be found in Sabatti *et al.* (2008) (219).

5.2.1.1 Genotyping data

SNP quality control (QC) and clean-up of genotyping data were performed by Nikman Adli Nor Hashim, another PhD student in Professor Alex Blakemore's group, as part of his PhD studies. The genotyping quality control process applied a Hardy-Weinberg Equilibrium (HWE) cut-off at an overall HWE P > 1 x 10-4 cut-off and poor-quality SNPs with minor allele frequency (MAF) <5% and call rate <98% were excluded.

For NFBC1986, genotyping was by performed the by a collaborative effort from the Department of Medicine, Imperial College London, and High Throughput Genomics, Wellcome Trust Centre for Human Genetics, University of Oxford. using Illumina Infinium®HumanOmniExpressExome-8v1.2arrays (Illumina Inc., San Diego, California, USA) that contains the base content of the HumanOmniExpress Beadchip plus an additional 250K high-value exome content discovered through exome sequencings. The genotyping success rate for all SNPs was >95.0% and none of the SNPs deviated from Hardy-Weinberg equilibrium (all P-values>0.0001) (315).

5.2.1.2. Phenotypic traits

The 20-item Toronto Alexithymia Scale (TAS-20) had already been applied, at the age of 15-16 years to NFBC1986 participants, and at the ages of 31 and 46 years in NFBC1966 participants. The 13-item Hopkins Symptoms Checklist (HSCL-13) measured current depressive symptoms. The HSCL-13 scores were only available among NFBC1966 participants.

5.2.2 Genetic association analysis

Genetic association analyses were conducted for TAS-20 score and depressive symptoms (HSCL-13) as continuous traits, using the PLINK 2.0 software package. Association testing was carried out under an additive genetic model.

For significant variants (single nucleotide polymorphisms, or SNPs) associated with TAS-20 score or HSCL-13 scores, the SNPsnap web-based tool was used to match and annotate them to the European 1000 Genome Project reference (1000G Phase 3) (239). The 'functional mapping and annotation of genetic associations' tool browser, FUMA (240) and MAGMA (1) were used for post-GWAS results interpretation. FUMA provides a single platform for GWAS data visualisation, which combines with the MAGMA software tool that uses multiple regression model for gene-set analysis. Using the FUMA platform, I created Manhattan plots, quantile-quantile (Q-Q) plots and regional association plots for the TAS-20 and HSCL-13 score GWAS datasets.

Pairwise linkage disequilibrium (LD) statistics (r^2) for SNPs located within same chromosome were assessed using the FUMA (240) and SNiPA platform (241). Manhattan plots were created to visualise top hits from the GWASs with the X-axis, showing the genomic co-ordinates, and the Y-axis showing the negative logarithm (denoted by log-10(P)) of the associated SNP p-value. Similarly, association plots were used to visualise a limited section of the genome, with X-axis showing the position of the SNP of interest on the chromosome, and the Y-axis showing the negative logarithm of the associated SNP p-value. Linkage disequilibrium plots created by SNiPA show the correlation between a sentinel SNP and its surrounding SNPs. In this case, the Y-axis shows the correlation coefficient (r^2), while the X-axis shows the chromosomal position of each SNP. Each variant indicates its functional annotation in the LD plot (241).

5.2.3 Statistical analysis

Following a standard genetic approach, association testing proceeded via regression analysis carried out in PLINK 2.0 (236). SNP genotypes were coded as 0, 1 or 2 copies of the minor allele (A1). Linear regression was performed to test for genetic association with continuous total TAS-20 score and HSCL-13 score separately as quantitative measures for alexithymia and depressive symptom traits. As a standard practice, the linear regression model was adjusted with covariates (sex and additional subpopulation structures; PC1-PC7). To investigate possible interactions between genetics and sex for alexithymia and depressive symptoms traits, --gxe and --interaction PLINK commands were used respectively for each phenotypic trait.

5.3 RESULTS

5.3.1 GWAS for TAS-20 total score in NFBC1966 and NFBC1986

GWA analysis for TAS-20 total score in the NFBC1966 and NFBC1986 study populations included 5291 subjects (2554 males, 2737 females) at the age of 31 years and 3369 subjects (1651 males, 1718 females) at the age of 16 years.

The Q-Q plots of the GWAS TAS-20 results for NFBC1966 and NFBC1986 are shown in Figure 5.1. The top genotyped SNPs in either NFBC1966 or NFBC1986 (which were analysed separately) are listed in Table 5.1.

Figure 5.2 shows the Manhattan plot of GWAS for TAS-20 in the NFBC1986 dataset and only one SNP: rs7030942 (MAF=0.472) located on chromosome 9 within gene SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 2 (*SMARCA2)* passed the suggestive genome wide significance cut-off (316) ($P > 5 \times 10^{-8}$ and $P \leq 1 \times 10^{-7}$).

Figure 5.3 shows the Manhattan plot of GWAS for TAS-20 in the NFBC1966 dataset: there were two SNPS: rs2242223 (MAF= 0.239) and rs747013 (MAF= 0.217) that passed the suggestive genome-wide significance cut-off for gene-set analysis ($p= 2.92 \times 10^{-6}$). rs2242223 and rs747013 are located on chromosome 5 within synuclein alpha interacting protein gene (*SNCAIP*). The location on both SNPS: rs2242223 and rs747013 is shown in the regional plot (Figure 5.4). The direction of effect and effect sizes for both SNPs are in concordance ($\beta_{rs2242223}= 1.931$ and $\beta_{rs747013}= 1.941$). However, the genotypes for rs2242223 and rs747013 were not highly correlated (LD r^2 ~0.7, D'= 0.9524).

Table 5.1 GWAS top-hits for TAS-20 scores in the NFBC1966 and NFBC1986 cohorts.

Chr	SNP	MAF	Within/Nearest Gene	HG19 Genomic Position	Risk Allele	BETA	P-value	Cohort
5	rs2242223	0.239	*SNCAIP*	121761461	G	1.93	8.10×10^{-8}	NFBC1966
5	rs747013	0.217	*SNCAIP*	121832058	A	1.94	3.25×10^{-7}	NFBC1966
13	rs4770323	0.102	*RFESDP1*	23422753	T	2.35	1.41×10^{-6}	NFBC1966
12	rs306664	0.376	*PPFIBP1*	27698751	A	-1.47	1.40×10^{-6}	NFBC1966
1	rs2794651	0.248	*DAB1*	58807475	C	-1.57	1.66×10^{-5}	NFBC1966
1	rs17405754	0.494	*PALMD*	100107260	C	1.422	2.85×10^{-5}	NFBC1966
22	rs624100	0.40	*SERPIND1*	21001422	T	-1.474	2.94×10^{-5}	NFBC1966
5	rs1946649	0.050	*SNCAIP*	121726714	G	1.561	3.04×10^{-5}	NFBC1966
9	rs16936951	0.462	*ADAMTSL1*	18657061	G	3.672	3.06×10^{-5}	NFBC1966
12	rs10047556	0.462	*DDX47*	12985907	A	1.427	3.30×10^{-5}	NFBC1966
9	rs7030942	0.472	*SMARCA2*	1610861	A	1.19	1.41×10^{-7}	NFBC1986
9	rs1853418	0.484	*SMARCA2*	1598874	G	1.16	2.96×10^{-7}	NFBC1986
9	rs702160	0.449	*SMARCA2*	1603814	A	1.10	1.21×10^{-6}	NFBC1986
9	rs9298744	0.448	*SMARCA2*	1599200	C	1.09	1.85×10^{-6}	NFBC1986
7	rs7806458	0.374	*TMEM176A*	150476888	G	-1.01	4.08×10^{-6}	NFBC1986
9	rs771667	0.497	*SMARCA2*	1565742	G	1.03	4.58×10^{-6}	NFBC1986
17	rs1849733	0.488	*SLFN12*	33749919	A	-0.98	5.94×10^{-6}	NFBC1986
9	rs697047	0.468	*SMARCA2*	1607940	A	1.00	8.5×10^{-6}	NFBC1986
5	rs7444241	0.245	*FASTKD3*	8265008	G	-1.13	1.18×10^{-5}	NFBC1986
5	rs4640759	0.242	*FASTKD3*	8278166	A	-1.11	1.4×10^{-5}	NFBC1986

Minor allele frequency (MAF) value is from European population (CEU)
SNP was plotted with its chromosome position on human genome 19 (HG19) reference

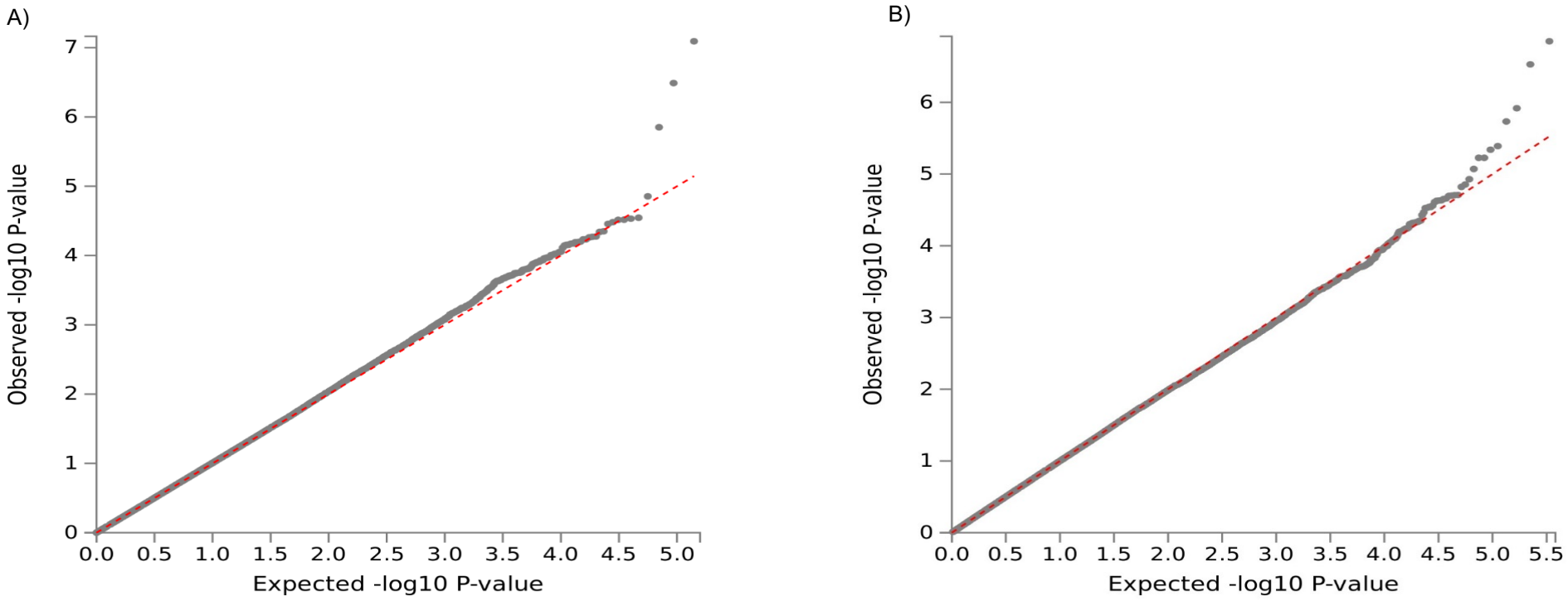

Figure 5.1 Q-Q plots of GWAS TAS-20 in NFBC1966 (A) and NFBC1986 (B) datasets. Plot compares observed −log10 p values of the tested SNPs on the vertical axis to expected values: log10 p values under the null hypothesis on the horizontal axis.

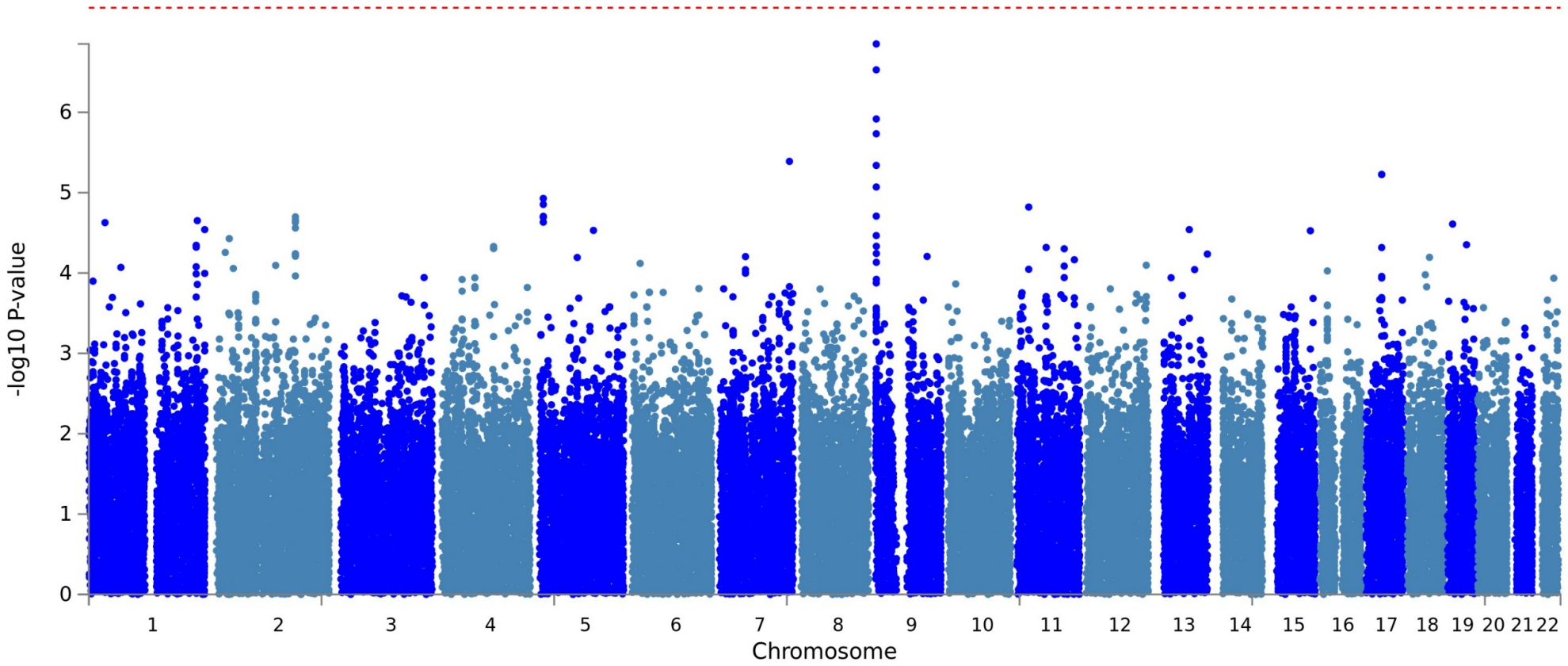

Figure 5.2 Manhattan plot of GWAS for TAS-20 at 16-year timepoint (NFBC1986). No SNPs passed the genome-wide significance cut-off for gene-set analysis by MAGMA that used multiple linear regression model (1).

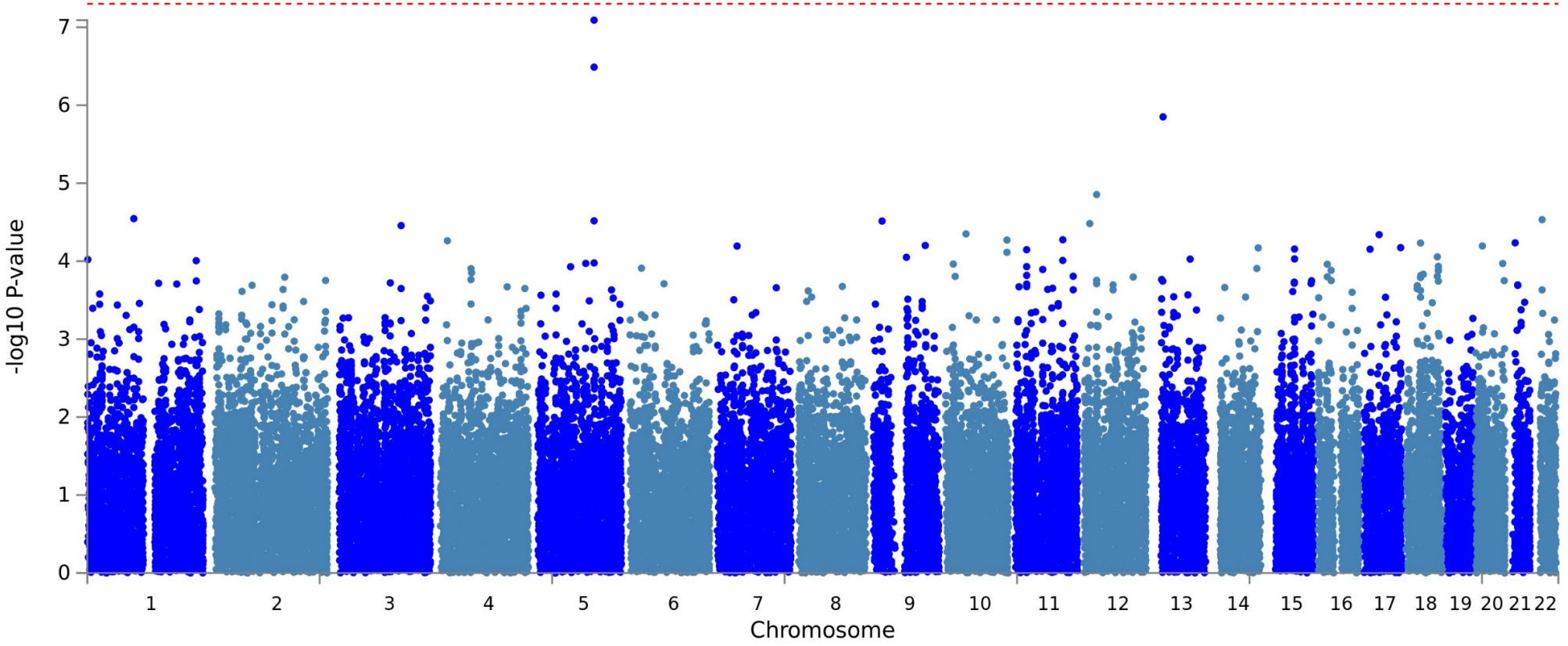

Figure 5.3 Manhattan plot from the GWAS summary statistics of the TAS-20 in the NFBC1966 cohort. Each point represents a SNP plotted with its chromosome position on human genome 19 (HG19) reference. Input SNPs were mapped to 17139 protein-coding genes and genome wide significance threshold was defined at $p < 5x10^{-7}$ (denoted as dashed red line in the plot).

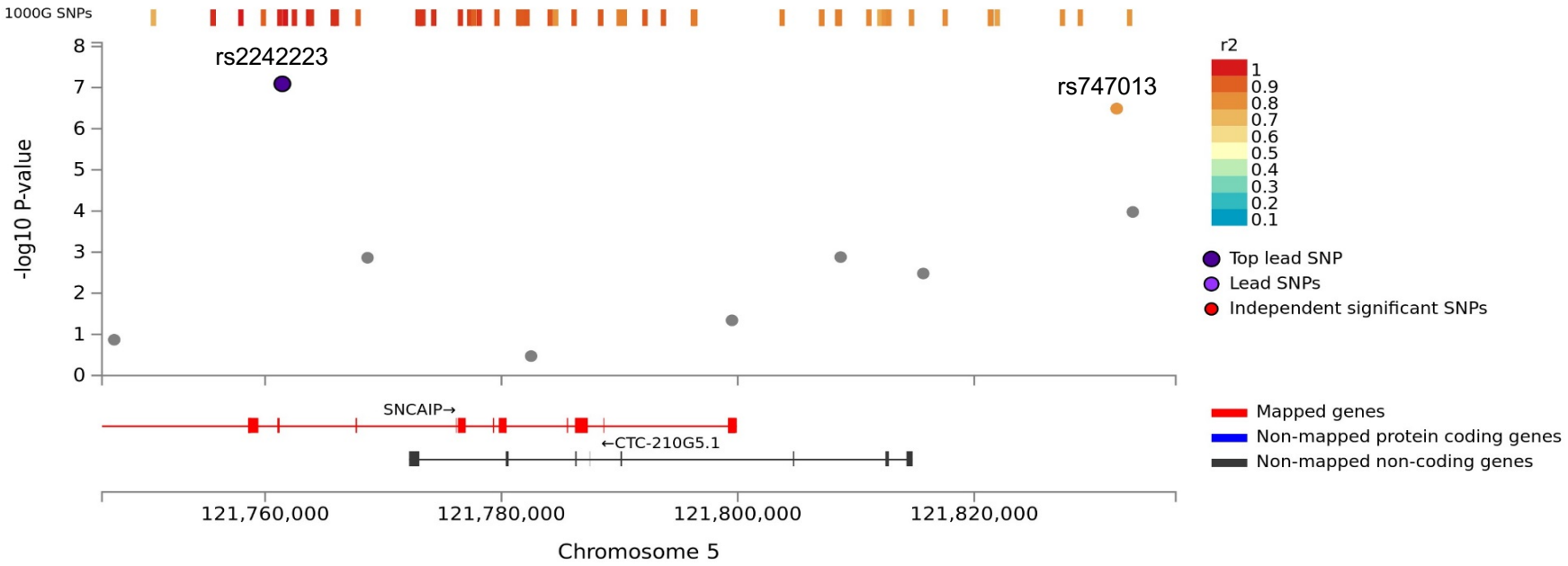

Figure 5.4 Regional association plot for the sentinel SNP, rs2242223 (*SNCAIP;* synuclein alpha interacting protein), in GWA analysis for TAS-20 score in the NFBC1966 dataset. The purple dot is the sentinel SNP or top lead SNP can be represented in linkage disequilibrium (LD) with other SNPs according to their pair wise correlation (r^2) test. The legend shows the details of color-coded SNPs and genes at the right panel. Grey coloured dots are SNPs that are not in LD of any of significant independent lead SNPs in the selected region. This plot was produced from FUMA platform.

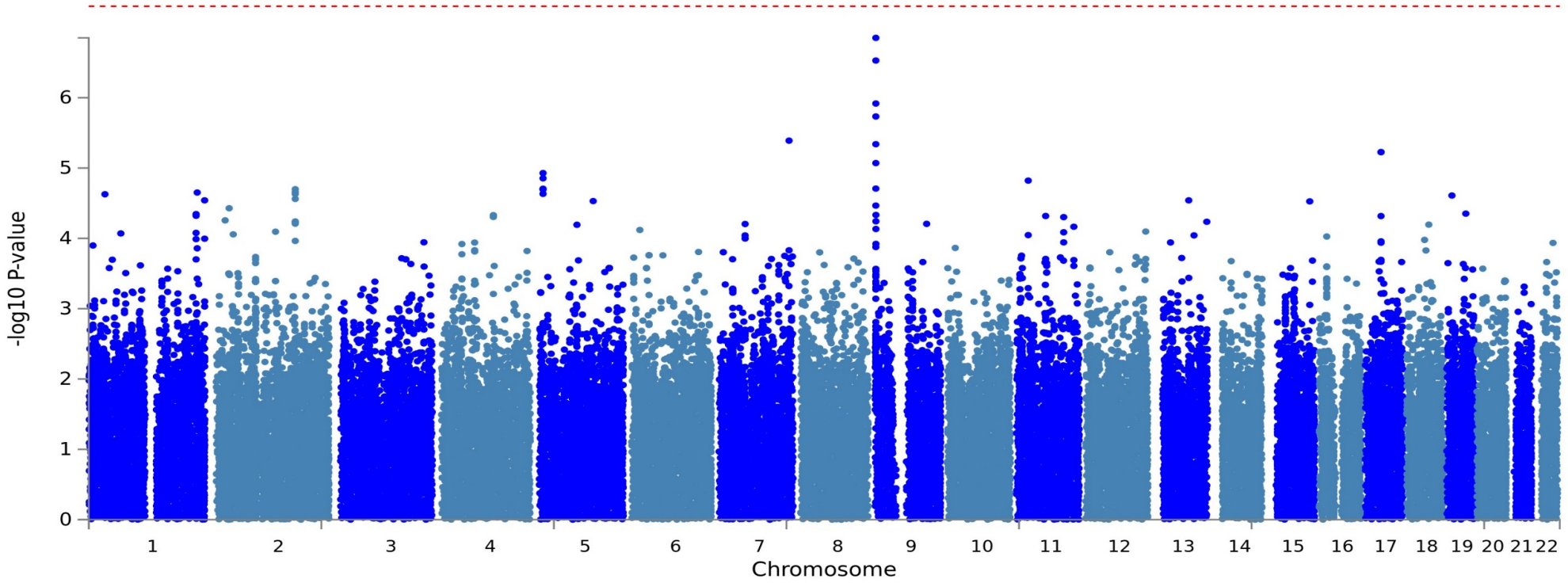

Figure 5.5 Manhattan plot from the GWAS summary statistics of the TAS-20 in the NFBC1986 dataset. Each point represents a SNP plotted with its chromosome position on human genome 19 (HG19) reference. Input SNPs were mapped to 17139 protein-coding genes and genome wide significance threshold was defined at $p < 5x10^{-7}$ (denoted as dashed red line in the plot).

5.3.2 GWAS for HSCL-13 score in NFBC1966

In this part of the research, I set out with the aim of assessing genetic factors influencing the psychological traits under study. I carried out GWAS for HSCL-13 score as continuous trait that measured current depressive symptoms. Few GWAS study have been conducted on depressive symptoms in the general population (317-321). However, most of the GWAS were conducted on major depressive disorder (MDD) (314, 322-328), due to the access and availability individual-level GWAS data from large consortia for eg; Psychiatric Genomics Consortium, UK BioBank, Generation Scotland: Scottish Family Health Study, and ENIGMA (Enhancing Neuro Imaging Genetics through Meta-Analysis) Network. Alexithymia and depression shared similar concepts or constructs of emotional processing deficits, so identifications of common genetic variants influencing both traits would enhance our understanding of the underlying biology of these traits.

The NFBC1966 genotype data is underpowered, so the results presented in this chapter must be considered as preliminary. The Q-Q plot and Manhattan plot of the GWAS HSCL-13 results for NFBC1966 are shown in Figure 5.6 and Figure 5.7, respectively. The top genotyped SNPs do not reach suggestive genome wide significance, as listed in Table 5.2. Also, none passed the cut-off for gene-set analysis by MAGMA.

The most significant SNPs were located on chromosome 1 [rs6424194 (MAF= 0.1461) and rs7546393 (MAF=0.1083)]: encoding are long noncoding RNA (lncRNA) variants which located on H3K27AC. LncRNA are generally named based on their relation to other known genes, and found throughout the genome. The lncRNA are small RNA molecule (200 base pairs) and transcribed in either proximal/cis or distal/trans regulation of the genes. H3K27AC has been found expressed in mouse livers (329) and marked as the potential enhancer in histone modifications (epigenetic mechanism).

Table 5.2 GWAS top-hits for HSCL-13 scores in NFBC1966 at age of 31 years.

Chr	SNP	MAF	Within/ Nearest Gene	HG19 Genomic Position	Risk Allele	BETA	P-value
1	rs6424194	0.1461	*H3K27Ac*	233583911	G	-0.04015	5.82×10^{-6}
9	rs4962069	0.4583	*C9orf171*	135390627	A	-0.03279	6.29×10^{-6}
1	rs7546393	0.1083	*H3K27Ac*	233597351	G	-0.04078	1.11×10^{-5}
2	rs6755244	0.09543	*PTPN18*	131148817	A	0.8976	1.37×10^{-5}
14	rs10134488	0.328	*NRXN3*	80252676	C	0.06493	1.80×10^{-5}
3	rs1993507	0.3767	*ZNF385D*	21684130	G	-0.03056	2.92×10^{-5}
20	rs6084644	0.1064	*SMOX*	4121799	C	0.02952	3.28×10^{-5}
19	rs352494	0.1511	*ANKRD24*	4185614	T	0.0494	3.55×10^{-5}
5	rs17499078	0.174	*NUDT12*	104292444	C	0.04236	3.75×10^{-5}
14	rs3784194	0.1461	*AKAP6*	32923626	T	0.04074	3.97×10^{-5}

SNP was plotted with its chromosome position on human genome 19 (HG19) reference.
Minor allele frequency (MAF) value is from European population (CEU)

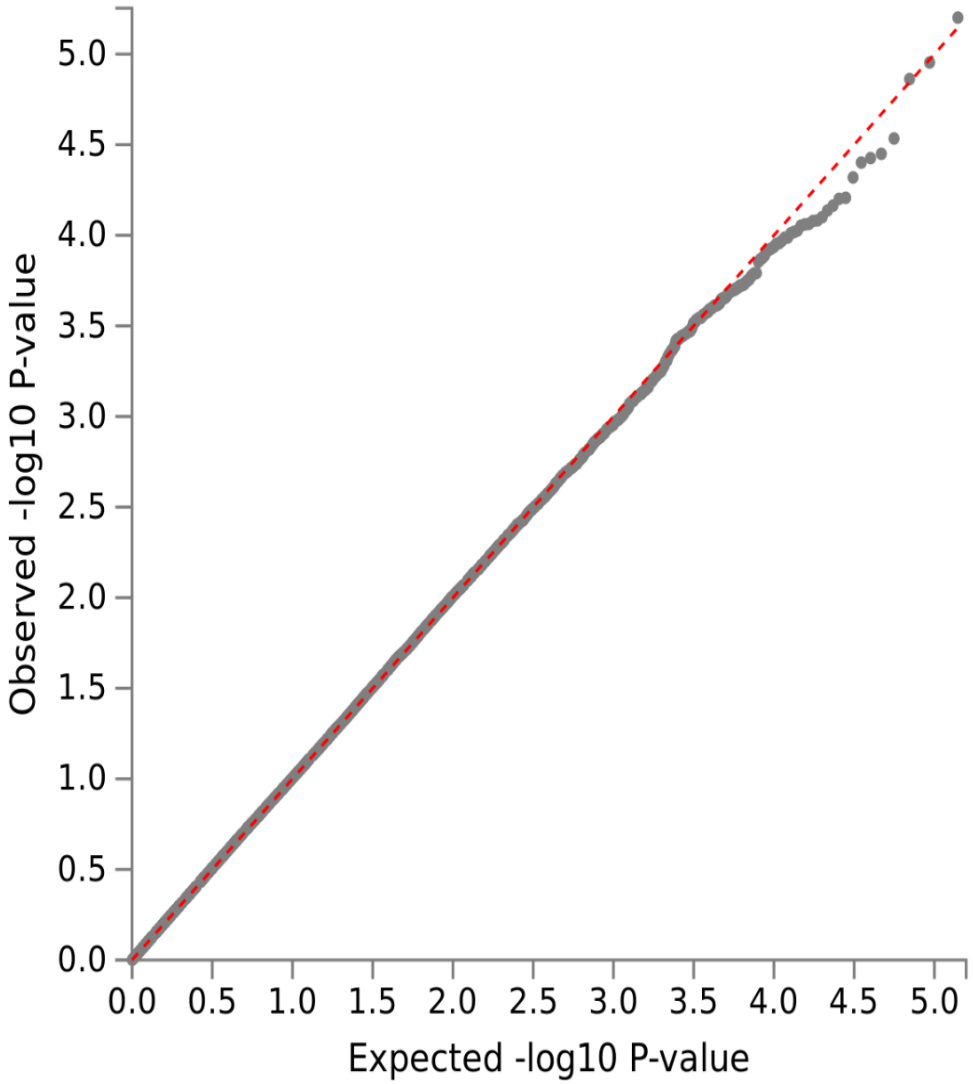

Figure 5.6 Q-Q plots of GWAS HSCL-13 in NFBC1966. Plot compares observed −log10 p-values of the tested SNPs on the vertical axis to expected: log10 p values under the null hypothesis on the horizontal axis

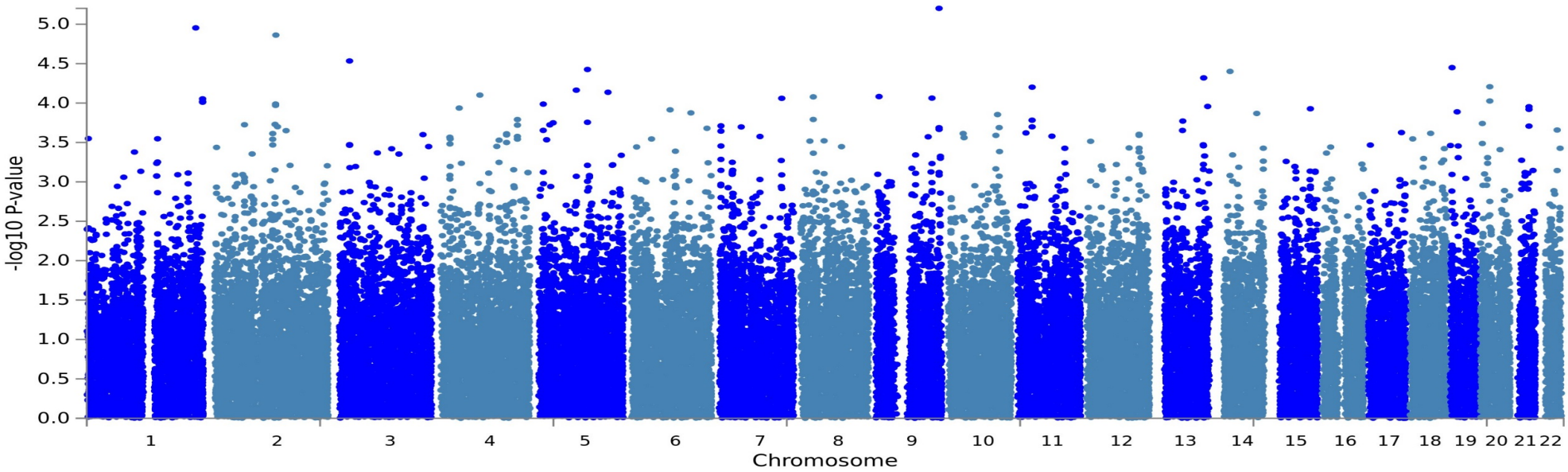

Figure 5.7 Manhattan plot from the GWAS summary statistics of the HSCL-13 in the NFBC1966. Each point represents a SNP plotted with its chromosome position on human genome 19 (HG19) reference. Input SNPs were mapped to 17139 protein coding genes.

5.3.3 Genetics and sex interaction for TAS-20 and HSCL-13 scores in NFBC1966

Alexithymia is more common among males (190) but depression prevalence is higher in females than in males (330). Sex differences and interactions were observed between TAS-20 and HSCL-13 scores in the NFBC1966 dataset, as described in Chapter 4 of this thesis.

Sex differences in general health, especially mental health, are not fully understood, due to the complexity of the traits, and little research has been done to explain them. Additionally, most mental health research neglects gene-by-sex interaction in the analytical models.

For gene × sex (gxs) interaction influencing TAS-20 scores, there are 20 SNPs with $p < 10^{-5}$ (Table 5.3). The top SNP, rs2076562 (MAF= 0.3827), which almost reached the suggestive genome wide significant level ($p= 9\times 10^{-7}$), is located at chromosome 17 within gene *SLC5A10* (solute carrier family 5).

Furthermore, four of the significant SNPs shown in Table 5.3 (rs12476979, MAF= 0.3529; rs6732726, MAF= 0.3539; rs11674694, MAF= 0.3529; rs4851487 MAF= 0.4245) are in LD ($r^2 \geq 0.8$), showing gxs interaction with TAS-20 scores located near a gene known to be associated with metabolic traits and with depression: *MAP4K4* (Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4) (331, 332). Linkage disequilibrium (LD) plots for the significant gxs interaction variants influencing TAS-20 is shown in Figure 5.8.

Table 5.3 Significant SNPs for gene × sex interaction influencing TAS-20 score.

Chr	SNP	MAF	Within/Nearest Gene	β_1	β_2	Z score	*Asymptotic P-value
17	rs2076562	0.3827	*SLC5A10*	2.45 (±0.56)	-1.00 (±0.43)	4.912	9.0×10^{-7}
2	rs12476979	0.3529	*MAP4K4*	-2.51 (±0.60)	0.89 (±0.46)	-4.523	6.1×10^{-6}
2	rs6732726	0.3539	*MAP4K4*	-2.47 (±0.60)	0.9 (±0.45)	-4.501	6.76×10^{-6}
7	rs1525463	0.4443	*TPK1*	-1.43 (±0.60)	1.76 (±0.44)	-4.416	1.01×10^{-5}
2	rs11674694	0.3529	*MAP4K4*	-2.37 (±0.60)	0.94 (±0.46)	-4.394	1.11×10^{-5}
2	rs6749889	0.163	*TSN*	-2.85 (±0.75)	1.26 (±0.57)	-4.357	1.32×10^{-5}
2	rs6541855	0.163	*TSN*	-2.85 (±0.75)	1.25 (±0.57)	-4.356	1.32×10^{-5}
15	rs6576648	0.4821	*NDN*	1.25 (±0.55)	-1.67 (±0.42)	4.247	2.17×10^{-5}
2	rs1448212	0.2922	*REG1B*	2.19 (±0.67)	-1.40 (±0.51)	4.246	2.17×10^{-5}
3	rs6445345	0.4463	*SYNPR-AS1*	1.90 (±0.55)	-1.03 (±0.42)	4.231	2.32×10^{-5}
3	rs6782747	0.2386	*RPL21P41*	-2.47 (±0.65)	0.99 (±0.50)	-4.216	2.49×10^{-5}
14	rs221697	0.06262	*DHRS2*	-5.63 (±1.67)	3.64 (±1.43)	-4.213	2.52×10^{-5}
18	rs1519143	0.2008	*CHST9*	-2.26 (±0.72)	1.53 (±0.55)	-4.189	2.81×10^{-5}
8	rs10089694	0.3539	*CSMD1*	-1.78 (±0.57)	1.22 (±0.43)	-4.188	2.81×10^{-5}
4	rs1020909	0.3072	*RASGEF1B*	-2.53 (±0.61)	0.68 (±0.47)	-4.147	3.37×10^{-5}
5	rs336178	0.166	*FASTKD3*	2.58 (±0.85)	-1.89 (±0.66)	4.144	3.42×10^{-5}
21	rs7278735	0.163	*OLIG1*	-2.41 (±0.69)	1.20 (±0.55)	-4.092	4.28×10^{-5}
2	rs4851487	0.4245	*MAP4K4*	-2.32 (±0.58)	0.65 (±0.44)	-4.082	4.47×10^{-5}
12	rs4883421	0.1511	*APOBEC1*	-2.93 (±0.74)	0.87 (±0.58)	-4.066	4.78×10^{-5}
20	rs2273137	0.07157	*NOP56*	-3.09 (±0.94)	1.75 (±0.73)	-4.06	4.9×10^{-5}

Minor allele frequency (MAF) value is from European population (CEU)

β_1 Regression coefficient in male group

β_2 Regression coefficient in female group

Z score, test for interaction

*Gene x sex interaction test

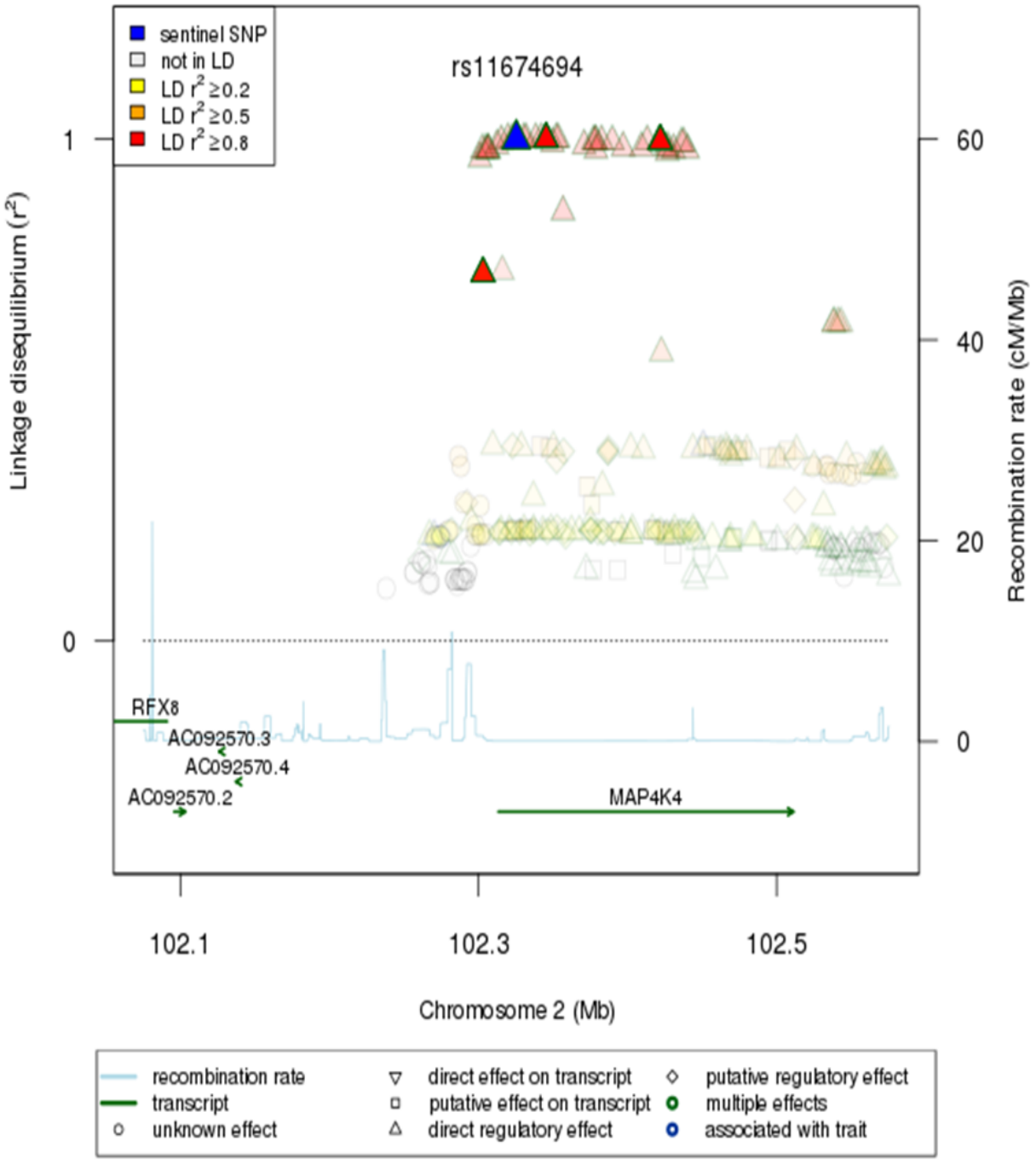

Figure 5.8 Linkage disequilibrium plot for significant gene × sex interaction SNPs influencing TAS-20 score located within *MAP4K4* (Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4) gene. This plot was produced from the SNiPA platform.

The *MAP4K4* gene product is involved in immune function (333) and has been previously associated with the aetiology of major depressive disorder (MDD) (334). Additionally, it was hypothesised that *MAP4K4* could play a mechanistic role in obesity-associated inflammation (335).

Several studies provide evidence that protein kinases, including mitogen-activated protein kinase (*MAPK*) (336, 337), play an important role in the formation of emotional memory in animals. In a mouse model, the gene ontology of *MAP4K4* gene includes protein metabolic process, response to stimulus, signalling and system development (338).

With regard to the role of sex in emotional processing, there were differences between the sexes in emotional appraisal in neurobehavioral studies (339, 340). Brain activations are stronger in females than males during processing of negative emotions.

Four SNPs located within *MAP4K4* (Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4) gene show gxs interaction influencing TAS-20 score in our current NFBC1966 dataset. This may suggest that MAP4K4 gene variation is important in an interaction between emotional processing deficits and sex.

As shown in Table 5.4, 15 SNPs were implicated in gxs interactions influencing HSCL-13 scores. The top SNP (rs5906144, MAF= 0.456) is located in non-coding DNA (LOC401585) on chromosome 23, and three SNPs (rs4978512, MAF= 0.3698; rs10121961, MAF= 0.3268; rs991897, MAF= 0.4225) are within the gene *SLC46A2* (solute carrier family 46 member 2). A linkage disequilibrium (LD) plot for the variants showing significant gxs interaction influencing HSCL-13 scores is shown as Figure 5.9.

Table 5.4 Significant SNPs for gene × sex interaction influencing depressive symptoms (HSCL-13 score).

Chr	SNP	MAF	Within/Nearest Gene	β_1	β_2	Z score	*Asymptotic P-value
23	rs5906144	0.456	*LOC401585*	-0.02 (±0.01)	0.04 (±0.01)	-4.503	6.71×10^{-6}
9	rs4978512	0.3698	*SLC46A2*	-0.02 (±0.01)	0.04 (±0.01)	-4.328	1.51×10^{-6}
9	rs10121961	0.3628	*SLC46A2*	-0.02 (±0.01)	0.04 (±0.01)	-4.296	1.74×10^{-6}
1	rs859395	0.4036	*TNR*	-0.04 (±0.01)	0.02 (±0.01)	-4.254	2.10×10^{-5}
9	rs991897	0.4225	*SLC46A2*	-0.03 (±0.01)	0.04 (±0.01)	-4.253	2.11×10^{-5}
6	rs2499615	0.3101	*HDAC2*	-0.03 (±0.01)	0.03 (±0.01)	-4.224	2.40×10^{-5}
19	rs278233	0.1402	*TSHZ3*	0.04 (±0.01)	-0.06 (±0.01)	4.209	2.57×10^{-5}
6	rs9320486	0.4423	*HS3ST5*	-0.04 (±0.01)	0.02 (±0.01)	-4.209	2.57×10^{-5}
6	rs1543991	0.2455	*CDK19*	0.03 (±0.01)	-0.04 (±0.01)	4.194	2.75×10^{-5}
14	rs755777	0.162	*PNP*	0.04 (±0.01)	-0.04 (±0.01)	4.18	2.91×10^{-5}
11	rs640927	0.1809	*MAP6*	0.04 (±0.01)	-0.03 (±0.01)	4.155	3.25×10^{-5}
23	rs5925649	0.4284	*METTL15P3*	-0.0004 (±0.01)	0.06 (±0.01)	-4.152	3.29×10^{-5}
5	rs6869426	0.2932	*IRGM*	-0.02 (±0.01)	0.03 (±0.01)	-4.143	3.42×10^{-5}
3	rs11711838	0.1083	*FGF12*	-0.03 (±0.01)	0.04 (±0.01)	-4.081	4.49×10^{-5}
5	rs256241	0.3698	*FSTL4*	0.02 (±0.02)	-0.09 (±0.02)	4.073	4.65×10^{-5}

Minor allele frequency (MAF) value is from European population (CEU)
β_1 Regression coefficient in male group
β_2 Regression coefficient in female group
Z score, test for interaction
*Gene x sex interaction test

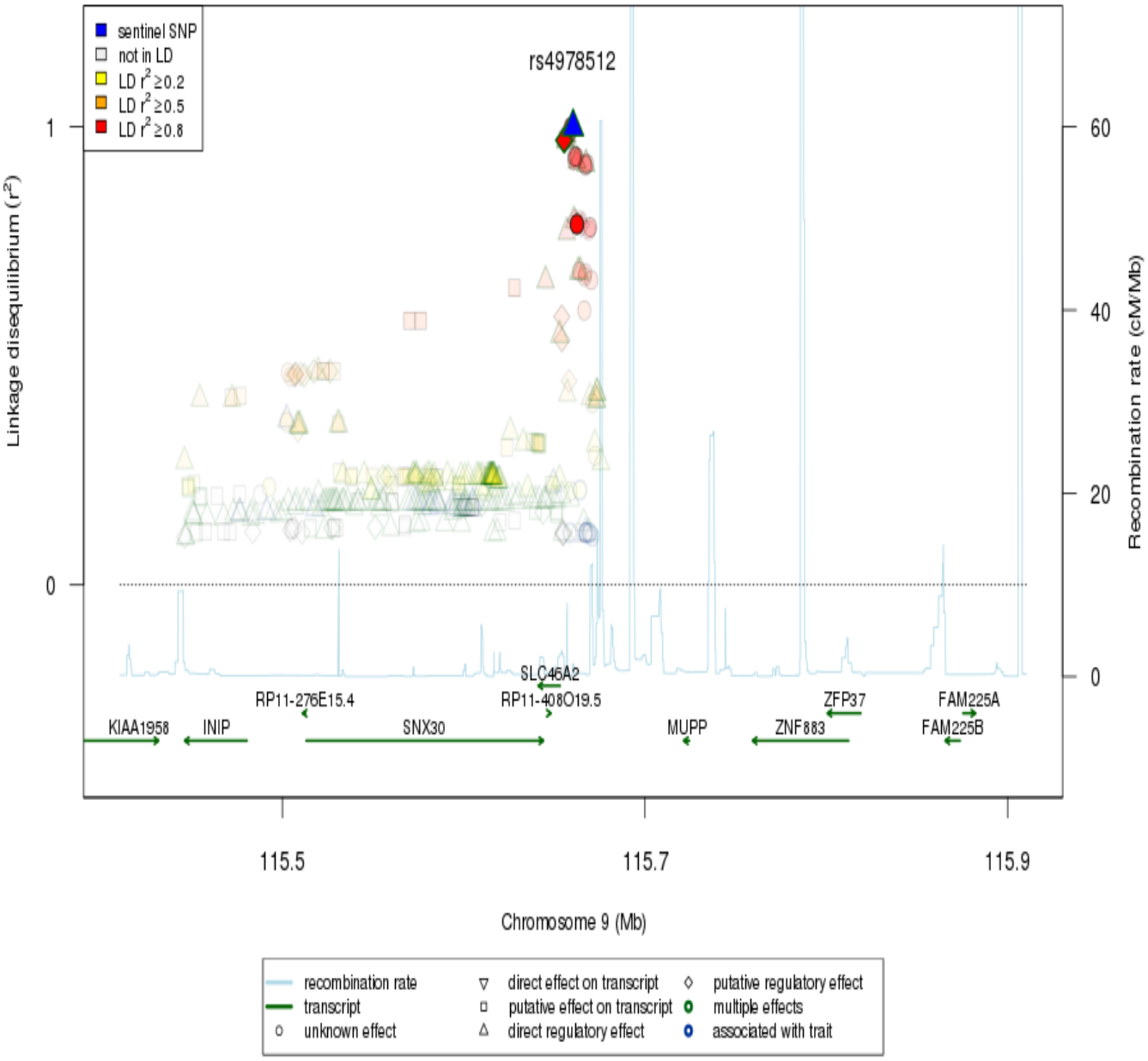

Figure 5.9 Linkage disequilibrium plot for significant gene × sex interaction SNPs influencing HSCL-13 scores located within the *SLC46A2* (solute carrier family 46 member 2)gene. This plot was produced from SNiPA platform.

5.4 DISCUSSION

In this chapter, I describe the identification of genomic loci associated with TAS-20 score and HSCL-13 score, as well as those interacting with sex to influence those phenotypic traits. Alexithymia and depression share a number of key features in the emotional processing deficits construct. In longitudinal analyses within the same individual over a 15-year period, alexithymic individuals had higher prevalence rate of current depressive symptoms, especially in males.

The relationship of genetic and environmental factors with alexithymia has previously been explored in three twin studies in different populations: the authors concluded that alexithymia is genetically influenced, with heritability estimates between 30-33% (10-12). Previous candidate gene-based association studies suggested that single nucleotide polymorphisms (SNPs) in four different gene/pathways are associated with the TAS-20 scores(82, 84, 310). These findings include *BDNF Val66Met* (rs6265), *DRD2/ANKK1 Taq1A* (rs1800497), *COMT Val108/158Met* (rs4680) and *5-HTTLPR* (rs25531).

In a more hypothesis-free approach, Mezzavilla *et al.* (2015) discovered another four significant novel SNPs (in *ABCB4*, *TP53AIP*, *ARHGAP32* and *TMEM88B* genes) that were associated with alexithymia status (TAS-20≥ 61) (311). For example, *ARHGAP32* also known as Rho GTPase activating protein 32, is located in chromosome 11, within a critical region for genes associated with autism spectrum disorder (ASD) (341). Deletion of the chromosomal region containing *ARHGAP32* was also associated with Jacobsen syndrome (342). People affected by Jacobsen syndrome present with 'autistic' features or symptoms, characterised by impaired cognition and social skills. This work differed from the analysis presented here because the genotyping array used (using Illumina exome chip, version Human Exome 12v1,

Illumina Inc) was not a GWAS chip, and was heavily biased towards functional variants in coding sequences.

In our GWAS analysis of TAS-20 score, one SNP, rs2242223 ($p= 8.10 \times 10^{-8}$) located at 5q23.2 within an intron of the *SNCAIP* (synuclein alpha interacting protein) gene, nearly approached the threshold for genome-wide significance ($p< 5 \times 10^{-8}$). The odds ratio, for Parkinson's disease, of rs2242223 is 1.01 [0.80–1.27] according to allelic association analysis by Haploview 3.2 software package (343). The synphilin-1 (SNCAIP) gene has been associated with increased risk of Parkinson's disease in three independent linkage studies (344-346).

Alexithymia is recognised as a primary characteristic of Parkinson's disease (347). A family study from the German population has provided compelling evidence that rs2242223 was inherited from a common ancestor (348). Functional studies have shown that α-synuclein (with which the *SNCAIP* gene product interacts) is involved in accelerating cellular dopamine uptake, and mutation of the gene encoding it is the most common cause of hereditary Parkinson's disease (225).

Another SNP that almost reached the genome-wide significance level was rs2076562 ($p= 9 \times 10^{-7}$) which was also significant in our gene x sex interaction analysis. rs2076562 is located at 17p11.2 within an exon of *SLC5A10*: the variant is predicted to be synonymous (does not alter amino acid sequence of the protein). Li *et al.* (2017) reported that novel loci near the *SLC5A10* gene were associated with metabolism. One SNP (rs117355297) showed genome-wide significant associations ($p< 5 \times 10^{-8}$) among European ancestry participants, with a biomarker of diabetes (1,5-anhydroglucitol) (349).

Other transporter genes of the human solute carrier (SLC) gene superfamily have been implicated in ASD. The contribution of each SLC gene; *SLC6A4*, *SLC9A6*, *SLC9A9*, *SLC1A2* to autism depends on the specific role of the gene product (350). Deletion of *SLC6A4* variants

has been associated with increased psychological sensitivity to stress, including depression and anxiety (351). The *SLC6A4* gene primarily functions in the central nervous system and encodes a serotonin (5-hydroxytryptamine, 5-HT) transporter.

Furthermore, a serotonin candidate-gene based study suggest a link with alexithymia and emotional processing deficiency (310). However, there are no previous reports of association of *SLC5A10* variants with any neuropsychiatric disorders. Our results suggest that rs2076562 within an exonic region of *SLC5A10* shows gene x sex interaction influencing TAS-20 score. Males show a positive association meanwhile females show a negative association between rs2076562 and TAS-20 score. Four significant SNPs (rs12476979, rs6732726, rs11674694, rs4851487) are located within the *MAP4K4* shows gene x sex interaction influencing TAS-20 score. Females show a positive association meanwhile males show a negative association between the four SNPs and TAS-20 score. These data may suggest that *SLC5A10* and *MAP4K4* gene variants are implicated in interaction between emotional processing deficits and sex.

There is emerging evidence that lncRNA variants may be implicated in ASD (352, 353) and depression (354-356). We found two SNPS located in a lncRNA (rs4978512 and rs10121961) that were associated with gene-sex interaction influencing HSCL-13 score in NFBC1966. According to a review on the genetics of depression, by Mullins and Lewis (2017) (119), multiple loci associated with depression have been reported in Chinese and European populations. Recently, a GWAS of major depressive disorder was published using data from the UK BioBank (357, 358), which sheds more light on the biological pathways underlying what is the commonest mental illness. Thanks to the increased power of such ultra-large-scale GWASs, the genetic architecture of depression will soon be unravelled and results can be translated to provide new biomarkers and therapeutic targets (119).

Despite the fact that GWAS methodology has provided valuable insights into disease biology, with more than 2,000 common disease-associated variants identified (67), there are issues of interpretation of these findings in any clinical context. The predictive value of even the most strongly-associated individual SNPs is very poor, and this is still only moderate when information from several genome-wide significant SNPs are combined into genetic risk scores (GRS). A more informative approach may be to use all of the GWAS information, including a much larger number of SNPs that modify risk, regardless of whether their association reached "genome-wide significance". Information from such polygenic risk scores (PRS), derived from the weighted sum of risk alleles with the weights specified by association coefficients (359), can be used to explore the utility of GWAS data in prediction of traits and/or diseases with a genetic component.

To our knowledge, this is the first GWA or genetic analysis of alexithymia. With the total genotypic and phenotypic NFBC1966 and NFBC1986 participants data that we had, we gained the power of more than 80% at $\alpha = 5\%$ level (under an additive genetic model) for linear regression, as assessed using the GCTA-GREML power calculator tool (360). Based on the GCTA-GREML results, the heritability (h^2) of alexithymia is 30-33%. Our study contributes to the ongoing worldwide effort to identify the genetic factors that affect alexithymia. We provide analysis in a homogenous Finnish birth cohort: the variants identified in this population could assist in understanding the underlying neurobiology of emotional constructs across different general and clinical populations.

These findings are in the discovery phase, and replication studies are warranted in other general populations with additional samples. Previous chapters have evidenced significant associations between alexithymia, depression and obesity. Thus, future GWAS meta-analyses for TAS-20

scores are suggested to provide sufficient power to allow adjustment for socio-economic factors and obesity.

CHAPTER 6 Alexithymia and Related Psychological Measures among UK Bariatric Surgery Patient

6.1 INTRODUCTION

In the previous chapters, the investigation of alexithymia (measured by TAS-20) and depression (measured by HSCL-13) in relation to body mass index and obesity of a general population, was described. In this chapter, a clinical severe obesity cohort, named Personalised Medicine of Morbid Obesity (PMMO), was used to investigate alexithymia, and depressive symptoms in relation to baseline characteristics and bariatric surgery outcomes.

Bariatric surgery is an effective treatment for morbid obesity (361). Despite successful weight-loss and improvement of co-morbidities, such surgery also carries risks, including potentially having a negative impact on psychological health outcomes such as depression, as well as increase in maladaptive behaviours, including alcohol abuse (362), and body image dissatisfaction (145, 363). Successful bariatric surgery outcomes, such as long-term weight loss, improvement of lipid profile, cardiovascular risk factors and type 2 diabetes mellitus (T2DM), are largely dependent on individual differences in factors such as genetic background, social and environmental exposures. In addition, psychological traits such as anxiety and depression are significant predictors for weight control outcomes (364). The expected large reduction in weight after surgery may contribute to concomitant changes in psychological health or behaviour, but more research on predictive factors is required. In this chapter, I explore TAS-20 scores among severely obese people before and after bariatric surgery, seeking to understand whether the scores correlate with any particular features of the patient group, or predict weight loss after surgery. Of particular note here is that obese individuals with Binge Eating Disorder (BED) have been suggested to have a distinct neurobiological phenotype: emotion processing deficit (365, 366).

A range of theories of eating behaviour, such as psychosomatic, internal and external, and restraint hypotheses have been posited in order to understand the role of emotion processing in obesity and/or eating disorders (200). However, the exact mechanism by which emotions affect eating behaviours and/or obesity, remains unknown. In addition, the exact roles of psychological disorders, anxiety and depressive symptoms in the aetiology of obesity are still questionable. Even less well-understood is the effect of deficits of emotional processing /alexithymia on this.

According to Pinna *et al.* (2011) and Leehr *et al.* (2015), several theories have been offered to explain the associations between alexithymia, anxiety, depression and emotional eating (22, 367). In line with previous debates on alexithymia stability, they suggested that alexithymia could be a primary (personality) or secondary (or state) trait connected to some cases of obesity. If alexithymia is a personality/primary trait for people with obesity risk factors (genetic and environmental), anxiety and depression could be acting as mediators for emotional eating, which could increase their food consumption and lead to obesity.

In a previous chapter, I demonstrated that, at least in the Northern Finnish Birth Cohort 1966, TAS-20 scores and alexithymia state are not constant over a 15-year study period and that variation in TAS-20 score may be related to BMI change over the same period. Here, I examine a small group of individuals with data both before and after bariatric surgery to investigate whether this holds true in severely-obese people experiencing weight loss after this intervention.

6.1.1 Aims of the study

i. To study the relationship between TAS-20 score, and depression among adults in a clinical bariatric surgery cohort, using cross-sectional and longitudinal designs.

ii. To investigate the possible relationship between TAS-20 scores and bariatric surgery outcomes in the same cohort.

I aimed to address the following research questions:

i. What is the prevalence of alexithymia, and depressive symptoms in our clinical bariatric surgery cohort?
ii. Is alexithymia associated with clinical characteristics of obese people before and/or after surgery (e.g. weight-loss percentage, bariatric surgery types, etcs)
iii. What other factors (sex, ethnicity, age, etcs) were associated with BMI before and/or after surgery?

6.1.2 Null hypotheses

i. There are no associations between pre-surgery TAS-20 scores, and depressive symptoms among adults in an obese clinical cohort. TAS-20 scores are higher among obese with depression than among those without these conditions.
ii. Alexithymia does not associate with weight loss trajectories and response to the therapeutic intervention of bariatric surgery. Subjects with alexithymia do not have poorer response to such intervention.

6.2 METHODS

6.2.1 Study design

Data for this chapter came from a prospective observational clinical trial of research participants with obesity (PMMO). This study was approved by the National Research Ethics Service (NRES) Committee London—Fulham and Riverside (REC reference 11\LO\0935 and study number 07/Q0411/19) and was performed in accordance with the principles of the

Declaration of Helsinki. The clinical trial started in November 2011 and Professor Alexandra Blakemore is the principal investigator of this project.

Further descriptions of the PMMO research study and its data collection were described in detail by Alsters (2016) in her PhD dissertation (221). Briefly, trained specialist nurses or clinical trial coordinators at various recruitment sites (Imperial College NHS Weight Centre, Chelsea Westminster Hospital NHS healthcare centre, Charing Cross Hospital, City Hospitals Sunderland and general practitioner surgeries) interviewed each research participant face-to-face, and written informed consents were obtained from all participants. Anthropometric data was measured upon recruitment (visit 1) and at every follow up visit; day of the surgery (visit 2), and two days (visit 3), 10 days (visit 4), six months (visit 5), 12 months (visit 6) and 18-24 months (visit 7) after surgery. If weight or other necessary data (such as sex, date of birth, psychological data, etc) were not available during follow-up visits, such data was obtained from the patients' hospital records, letters or online questionnaire set (Qualtrics). Research ethics amendments to apply the TAS-20 questionnaire to PMMO participants were approved in May 2015 and were successfully implemented in August 2015.

Research participants were obese adults (BMI >35 kg/m^2) aged between 18 and 65 years. For the purpose of this study, they were categorised into three groups, as described below:

Group 1 (before or no-surgery): Patients recruited at Imperial Weight Centre or other bariatric centres in the UK who either have completed TAS-20 questionnaires during visit 1, before having surgery or during visit 2, or who decided not to pursue bariatric surgery (n=133).

Group 2 (Post-surgery): Patients who completed TAS-20 questionnaires and had already had bariatric surgery (n=155).

Group 3 (Longitudinal): Patients who completed TAS-20 questionnaires before and after the surgery, and whose psychological questionnaires, clinical and demographic data were available in the PMMO database at the time of analysis (n=20).

6.2.2 PMMO clinical trial

As described in detail elsewhere (Alsters, 2016), an extensive range of biological specimens such as blood, saliva, faeces or tissue biopsies, as well as phenotypic variables including demographic information, family health history, anthropometric data, results of clinical psychological screening, and a range of questionnaires were collected at a single or multiple time points during the clinical trial period (221). An anonymised online PMMO server database (maintained by Centre for Integrative Systems Biology and Bioinformatics at Imperial College London) was used for PMMO data management. Electronic medical records from all recruitment sites were carefully reviewed, and data subsequently transferred into the PMMO database for systematic storage. Psychological questionnaire responses for this study were obtained via postal inquiries and/or an online survey tool through Qualtrics (Provo, UT, the United States). For data uniformity and quality control, completed psychological questionnaires were also electronically saved in the Qualtrics system.

For this study, data from the TAS-20 questionnaire (6, 7), and the Hospital Anxiety and Depression scale (HADS) (232) were selected for analysis. All PMMO participants were asked to complete the questionnaires package during visit 1 and visit 3 onwards (after surgery) at the clinic using online Qualtrics system. If the participants unable to complete the questionnaire during the visit, an e-mail link to answer the questionnaire in Qualtrics system was given to each participant along with their research number. If still no response in the Qualtrics system from post-surgery patients after at least 6 months, postal questionnaires were sent to their home

addresses. Additionally, a follow up Qualtric's e-mail link were given to the pre-surgery PMMO participants who attended visit 1 but yet to undergo bariatric surgery. PMMO participants (with available BMI data) who had completed TAS-20 and other-related psychological questionnaires before surgery, or were not having surgery (Group 1), and after surgery (Group 2) were included in this study.

6.2.3 Measures

Demographic and clinical data, such as age, sex, ethnicity, age of onset of obesity, and comorbidities (Diabetes Mellitus diagnosis, hypercholesterolemia, learning difficulties, congenital defects, maternal history of recurrent pregnancy loss, and psychiatric disorders) were collected via patient registration forms and recruitment interviews. Clinical assessment for psychiatric disorders, based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), was conducted by trained psychologists at bariatric surgery centres during visit 1. The psychiatric disorders that were directly assessed included BED, clinical depression, bipolar disorder, borderline personality disorder, schizophrenia, or any history of such disorders. Missing demographic data from the participants were retrieved from the PMMO database or NHS medical records. All recruited patients from 15th August 2015 until 9th January 2018 (N=500, based on online PMMO database) were asked to complete the questionnaires, and 57.6% (n=288) did so on at least one occasion.

Anthropometric data were measured during visit 1, as well as during follow-up visits (to obtain information for weight-loss trajectories following surgery). Weight loss was defined as percentage of initial weight which was lost (%WL), which was calculated by dividing the weight during visit 1 (as baseline), in kilograms, by the weight during each follow-up visit, in kilograms, multiplied by 100. Self-reported baseline and follow-up heights (cm) and weights

(kg) were also collected by questionnaires. BMI (kg/m^2) for each participant was calculated by dividing weight (kg) by height^2 (m^2).

The English version of the TAS-20 questionnaire was used (a translated version of the TAS-20 questionnaire had been used in the NFBC1966 and NFBC1986 cohorts). Alexithymia was defined as having a total TAS-20 score ≥61 points. Cronbach's alpha in the obese sample was 0.84 for the full TAS-20 scale, 0.85 for the subscale Difficulty Identifying Feelings (DIF), 0.79 for Difficulty Describing Feelings (DDF) and 0.69 for Externally Oriented Thinking (EOT) (62). TAS-20 change (ΔTAS-20) was calculated using the following equation:

$$\Delta\text{TAS-20} = \text{Total TAS-20 score}_{\text{(post-surgery)}} - \text{Total TAS-20 score}_{\text{(baseline)}}$$

6.2.4 Statistical Analysis

For all analyses, the Statistical Package for Social Sciences software (SPSS for Windows, version 22.0, 2004, Chicago, IL, USA) was used. Student's T-test (t), analysis of covariance (ANCOVA) and Pearson's chi-squared test (χ^2) were appropriately used to compare continuous and categorical variables, at cross-sectional and longitudinal design. As standard, ANCOVA models performed at the cross-sectional level were adjusted for covariates (sex, age, ethnicity, T2DM status and baseline BMI). The necessary adjustment variables were explored earlier from literature, in NFBC data sets and in the present study. Bonferroni adjustment was used to correct for multiple testing in main effects comparisons and interactions in ANCOVA models. In addition, surgery type, such as Roux-en-Y (RNY) gastric bypass, adjustable gastric band, and vertical sleeve gastrectomy were included as additional covariates when conducting an ANCOVA model among post-surgery participants, since these surgeries have different expected weight loss outcomes.

Comparative analysis was conducted between participants who answered questionnaires before and after surgery were analysed together (n=288) as the PMMO participants who completed the TAS-20 questionnaire, with available data at baseline. The participants who had their clinical/anthropometric data available to us, and who had completed the questionnaires at baseline (n=133) or after surgery (n=155), were analysed cross-sectionally.

Since the PMMO study is still ongoing, with quite a lengthy patient journey to surgery, and since the TAS-20 questionnaires were only implemented in late 2015, the sample size available to us for any longitudinal analyses with testing before and at 12 months post-surgery is still relatively small (n=20). There were also participants who were either lost to follow up due to various medical and personal reasons, and those who are still waiting for the surgery, or have missing data (e.g. sex, height and weight).

Normality, linearity, homoscedasticity, and multi-collinearity were assessed to ensure that assumptions of regression models were met. For multicollinearity, collinearity statistics were performed and absence of multi-collinearity was observed in the current analysis (Tolerance > 0.1 and VIF < 2) (234). The residuals were normally distributed and there was homoscedasticity in both pre- and post-surgery multiple liner regression analysis.

Adjusted R^2 with ±SEM (standard error of measurement) were calculated to assess the proportion of variability in the outcome (dependent) variables that is explained by predictors or independent variables in multiple regression models (i.e. variability in the outcome explained by the full model). Age, sex, ethnicity, T2DM status and baseline BMI were used as standard covariates in multiple linear regression models. The type of surgery was also included as an additional predictor in post-surgery regression models. Separate regressions were performed for each subscale (as an independent variable) in the TAS-20, and depression. These

were conducted to avoid violation of underlying assumptions of the regression models (multi-collinearity).

Associations of bariatric surgery outcomes (%WL, BMI, weight) and TAS-20, with related psychological measures before and after surgery, were assessed using linear regression models adjusted for age, sex, and ethnicity. Pearson's correlation (r) analyses were conducted to identify the relationships between the psychological questionnaire responses (TAS-20, and HADS) provided pre- or post-surgery (cross-sectional), as well as in a small subset of participants (n=20) who had completed TAS-20 questionnaires both pre- and post-surgery (longitudinal). The TAS-20, and HADS scores and their subscales (DDF, DIF, EOT, Anxiety, Depression) answered before (n=133), or after having bariatric surgery (n=155) were treated as continuous variables for Pearson's (r) correlation analysis.

6.3 RESULTS

6.3.1 Characteristics of the overall study group

Baseline obesity-related clinical and demographic characteristics of the subset of PMMO participants included in the present analysis are shown overall and by sex in Table 6.1. Sex differences were seen in ethnicity ($\chi_{(7)} = 18.36$, $p = 0.01$), age ($\chi_{(3)} = 14.89$, $p = 0.002$), T2DM status ($\chi_{(3)} = 13.64$, $p = 0.003$) and depression status ($\chi_{(3)} = 12.56$, $p = 0.006$). The mean age of study participants was 48 (±11.47) years. The majority of participants were British or Irish females (60.4%, n=124). Obesity onset was self-reported to have been at age <10 years and >10 years for 27.3% and 72.7% of the study sample, respectively.

Nearly 30% of the study sample had type 2 diabetes mellitus (T2DM) at baseline. The prevalence of early childhood onset of obesity and T2DM reported here were similar to the

overall PMMO participants (N=2412) with available clinical data in the online PMMO database, which were 20.3 (n=489) and 22.7% (n=548), respectively.

Eleven participants met the diagnostic criteria for current binge eating disorder at baseline, and 12 of the participants had a history of BED in previous 12- months. The prevalence of BED was 8% (n=23): the majority of participants with BED were in the post-surgery group (73.9% of BED patients, n=17). This BED prevalence rate is in line with that of all PMMO participants recruited at Imperial Weight Centre (N=2412) as of January 2018 which was 8.62% (n=212). No TAS-20 score, BMI or sex differences were seen among participants with BED diagnosis.

The prevalence rate of alexithymia was 21.2% [male: 26.1% (n=18), female: 19.6% (n=43)] in the current study ($\chi_{(1)} = 1.31$, $p = 0.25$). In addition, the prevalence of clinical depression, (current and past diagnosis) was 24.3% [(male: 31.9% (n=22), female: 21.9% (n=48)] ($\chi_{(1)} = 2.83$, $p = 0.09$). The mean ages of alexithymic and non-alexithymic participants in this study were 50.5 (±11.68) and 47.7 (±11.37) years, respectively: there was no difference in mean age between the two groups ($t_{(284)} = 2.80$, $p = 0.09$). However, sex differences were observed between age groups: male participants above 55 years old were more likely to have alexithymia than females or younger participants ($\chi_{(3)} = 14.36$, $p = 0.002$).

Overall, participants who had a history of clinical depression diagnosis had higher total TAS-20 score ($t_{(191)} = 3.19$, $p = 0.002$) and baseline weight ($t_{(281)} = 2.46$, $p = 0.02$) by 5.97 (± 1.87) and 10.45 (± 4.26) than participants who had no history of clinical depression diagnosis.

Table 6.1 Demographic and clinical characteristics of the PMMO participants who completed the TAS-20 questionnaire, with available data at baseline (n= 288).

Participant characteristics	**Overall Sample (n=288)**	**Males (n=69)**	**Females (n=219)**
Age, years (%)			
21-38	23	16.2	24.8
39-48	24.4	14.7	28.9
49-55	33.7	35.3	33.5
56-71	18.9	33.8	13.8
Age of onset obesity (%)			
<10 years old	27.3	26.2	27.6
>10 years old	72.7	73.8	72.4
Height, m (±SD)	1.67 (±0.1)	1.77 (±0.1)	1.64 (±0.1)
Weight, kg (±SD)	122.0 (±31.2)	131.4 (38.0)	119.1 (28.1)
BMI, kg/m^2 (±SD)	45.3 (±9.8)	44.2 (11.6)	45.7 (10.9)
Ethnicity (%)			
British/ Irish	60.3	70.6	57.1
Caribbean/African	9.0	-	11.5
Indian/ Pakistani/ Bangladeshi	7.2	8.8	6.5
Mixed background	4.8	5.9	4.6
Any other Caucasian background	11.7	4.4	14.3
Any other Asian background	1.0	1.5	0.9
Any other mixed background	1.0	-	1.4
Other	4.8	8.8	3.7
Diabetes status (%)			
Type 2 diabetes mellitus (T2DM)	22.8	40.3	17.3
Insulin-dependent T2DM	5.0	6.5	4.6
Impaired fasting glycaemia	18.1	8.1	21.3
Normal	54.1	45.2	56.9
Alexithymia status, TAS-20≥ 61 (%)	21.2	26.1	19.6
Binge eating disorder or history of binge eating disorder (%)	8.0	8.7	7.8
Clinical depression (%)	11.8	31.9	21.9
History of clinical depression (%)	11.1	3.1	8.0
History of psychiatric disease (other than depression) (%)	1.7	0.3	1.4

6.3.2 Comparative analyses between Group 1 and Group 2 participants

Table 6.2 shows the clinical and psychological characteristics of PMMO participant subgroups; Group 1 (pre- or no surgery, N= 133) and Group 2 (post-surgery, N= 155). There were 32 patients in Group 1 that had already had surgery at the time of analysis, but who had completed the questionnaires before surgery. Cross-sectional analysis was conducted separately for each group.

There was no difference in alexithymia prevalence or total TAS-20 scores between pre-and post-surgery groups. In addition, there were no significant associations between the total TAS-20 scores (or its subscales) and clinical variables (BMI at 12 months post-surgery, T2DM status and percentage weight loss) ($p > 0.05$). There were significant differences in mean total HADS scores ($t_{(181)} = 3.78$, $p < 0.0001$), HADS-Depression subscale ($t_{(194)} = 4.38$, $p < 0.0001$) between pre- and post-surgery groups.

Pre-surgery patients with alexithymia scored higher on the HADS-Anxiety and HADS-Depression subscales than those who did not have alexithymia by 7.43±3.58 ($t_{(90)} = 4.28$, $p < 0.0001$) and 6.21±2.41($t_{(91)} = 4.68$, $p < 0.0001$), respectively. Post-surgery participants with alexithymia (considered as a binary trait) had higher total HADS scores (20.95 ±7.74, $t_{(110)} = 6.29$, $p < 0.0001$), including both HADS-Depression (9.33 ±4.96, $t_{(100)} = 4.15$, $p < 0.0001$) and HADS-Anxiety (11.95 ±3.44, $t_{(109)} = 6.40$, $p < 0.0001$) compared to non-alexithymic post-surgery participants.

Table 6.2 Clinical and psychological measures of the PMMO participants by pre- and post-surgery groups with available data (n =288). Bold texts are for significant comparisons between pre-surgery (or not having surgery) and post-surgery groups.

Participant's characteristics	**Pre-Surgery (N= 133)**	**Post-Surgery (N=155)**	**P-value¥**
Male/ Female (%)	26.4/73.6	21.2/78.8	NS
Age, years (±SD)	46.8 (±11.80)	49.7 (±11.03)	NS
Baseline BMI, kg/m^2 (±SD)	46.0 (±7.63)	46.0 (±9.42)	NS
Weight, kg (±SD) (pre- or post-surgery)	**116.8 (±32.07)**	**93.9 (±19.79)**	**0.032**
Percentage of weight loss (±SD)	**-**	**24.30 (±14.20)**	**-**
Diabetes status (%)			NS
Type 2 diabetes (T2DM)	24.4	18.4	
Insulin-dependent T2DM	4.7	1.1	
Impaired fasting glycaemia	13.4	3.4	
Normal	57.5	77.0	
Type of surgery (%)¶			**<0.001**
RNY gastric bypass	8.3	53.2	
Gastric band surgery	2.8	9.6	
Vertical sleeve gastrectomy	11.1	37.2	
No surgery	77.8	-	
Early age of obesity onset € (%)	26.9	28	NS
Alexithymia status (%)	22.2	19.9	NS
Clinical BED diagnosis (%)	1.4	2.4	NS
History of BED diagnosis (%)	0.7	3.5	NS
Clinical current depression (%)	**6.3§**	**16.7§**	**0.001**
History of depression (%)	**4.9§**	**16.7§**	**0.002**
Psychological measures*			
Total TAS-20 score	50.83 (±11.76)	48.94 (±12.34)	NS
TAS-20- DDF	12.86 (±3.98)	12.51 (±4.44)	NS
TAS-20- DIF	18.27 (±6.06)	16.81 (±5.97)	NS
TAS-20- EOT	19.69 (±3.92)	19.62 (±4.30)	NS
Total HADS score	**16.90 (±7.98)**	12.58 (±8.09)	**0.003**
HADS- Depression	**8.51 (±4.17)**	6.11 (±4.31)	**<0.001**
HADS- Anxiety	8.77 (±4.52)	7.69 (±4.07)	NS

Data is presented as mean (±SD) for psychological measures. ¥Comparison analyses used 2-sided independent t-test for continuous variables and a chi-squared test between categorical variables. ¶N=32 in pre-surgery group already had surgery but have not completed questionnaire after surgery. €Obesity onset at <10 years old. §N=18 for pre-surgery and N=55 for post-surgery that had available clinical data on depression diagnosis. BED= Binge-Eating Disorder available clinical data (n =23). NS=not significant.

6.3.3 Correlational analysis between TAS-20 and HADS scores

Moderate correlations were seen between TAS-20 subscales (DDF and DIF) and HADS subscales (anxiety and depression) ($r_{(110)}$=0.49-0.60, $p<0.001$) among pre-surgery patients (Group 1). The EOT subscale of the TAS-20 showed the lowest significant correlation with HADS subscales when tested before surgery ($r_{(110)}$=0.25-0.26, $p<0.001$) (Table 6.3).

The same pattern was seen in post-surgery patients (Group 2): there were significant correlations between TAS-20 subscales (DDF and DIF) and HADS (Anxiety and Depression) subscales of post-surgery patients ($p<0.001$, Table 6.4). No significant correlation was seen between the EOT subscale of the TAS-20 and HADS-Depression subscale (Table 6.4).

Table 6.3 Correlation matrix between the subscales of TAS-20 and HADS among pre-surgery patients. Numbers 1-5 of the Pearson correlation matrix represent variables as shown in the table's first column.

Psychological measures	Mean (±SD)	Study sample (n)	Pearson correlations				
			1	2	3	4	5
1. TAS-20- DDF	12.86 (3.98)	110	1	0.72*	0.52*	0.60*	0.52*
2. TAS-20- DIF	18.27 (6.06)	110	0.72*	1	0.41*	0.53*	0.49*
3. TAS-20- EOT	19.69 (3.92)	110	0.52*	0.41*	1	0.25*	0.26#
4. HADS- Depression	8.51 (4.17)	112	0.60*	0.53*	0.25*	1	0.54*
5. HADS- Anxiety	8.77 (4.52)	113	0.52*	0.49*	0.26*	0.54*	1

*Correlation is significant at the 0.01 level #Correlation is significant at the 0.05 level.

Table 6.4 Correlation matrix between the subscales of TAS-20 and HADS among post-surgery patients. Numbers 1-5 of the Pearson correlation matrix represent variables as shown in the table's first column.

Psychological measures	Mean (±SD)	Study sample (n)	Pearson correlations				
			1	2	3	4	5
1. TAS-20- DDF	12.51 (4.44)	156	1	0.72#	0.49#	0.41#	0.49#
2. TAS-20- DIF	16.81 (5.97)	156	0.72#	1	0.42*	0.49*	0.61*
3. TAS-20- EOT	19.62 (4.30)	156	0.49#	0.42*	1	0.15	0.24#
4. HADS- Depression	6.11 (4.31)	102	0.41#	0.49*	0.15	1	0.66*
5. HADS- Anxiety	7.6 (4.07)	111	0.49#	0.61*	0.24#	0.66*	1

*Correlation is significant at the 0.01 level #Correlation is significant at the 0.05 level.

6.3.4 Bariatric surgery outcomes among after surgery participants

As expected, there was an association between BMI following surgery at 12 months and bariatric surgery type (bypass, band, and sleeve) ($F_{(2, 97)}$ =6.27, p= 0.003) as shown in Figure 6.1. ANCOVA revealed that associations between BMI following surgery (BMI-post at 12 months) and surgery types ($F_{(2, 33)}$ =4.18, p= 0.024), remained significant after adjustment for age, sex, ethnicity and T2DM status (after surgery). The significant association was only in females, but not males in the post-surgery group ($F_{(2, 22)}$ =4.04, p= 0.032). After the Bonferroni post hoc test, the mean BMI following surgery at 12 months was higher in participants who had undergone vertical sleeve gastrectomy surgery than in participants who had undergone gastric bypass surgery by 4.0 (±1.36) (p= 0.012).

Post-surgery's HADS-Depression was positively correlated with BMI following surgery, at 6 ($r_{(101)}$= 0.27, p= 0.005) and 18 months ($r_{(32)}$= 0.55, p= 0.001). Pre-surgery BMI data was available for most of participant in Group 2 (n=155): 60.26% (n=94), 64.10% (n=100), 67.74% (n=105), and 34.84% (n=54) post-surgery participants had attended follow-up visits at 10 days (visit 4), six months (visit 5), 12 months (visit 6) and 18-24 months (visit 7) after surgery, respectively (Figure 6.1). As expected, the percentage weight loss (%WL) at 18 months was higher in participants who had undergone gastric bypass surgery than in participants who had had placement of an adjustable gastric band, by 12.77 (±3.72) (p= 0.004).

Figure 7.1 shows the %WL trajectories in the post-surgery group: there was no significant sex difference in the mean %WL at either follow-up visit. Also, alexithymia status (TAS-20≥ 61) did not influence %WL trajectories in our dataset.Significant differences in mean BMI (as an intended result of the surgery) were expected, and seen, in pre-surgery BMI compared to post-surgery BMI at visit 5 (v5), visit 6 (v6) and visit 7 (v7) by 8.07 (±0.51) kg/m^2, 12.28 (±0.27) kg/m^2, and 13.92 (±0.85) kg/m^2, in Group 2 participants (all p< 0.001).

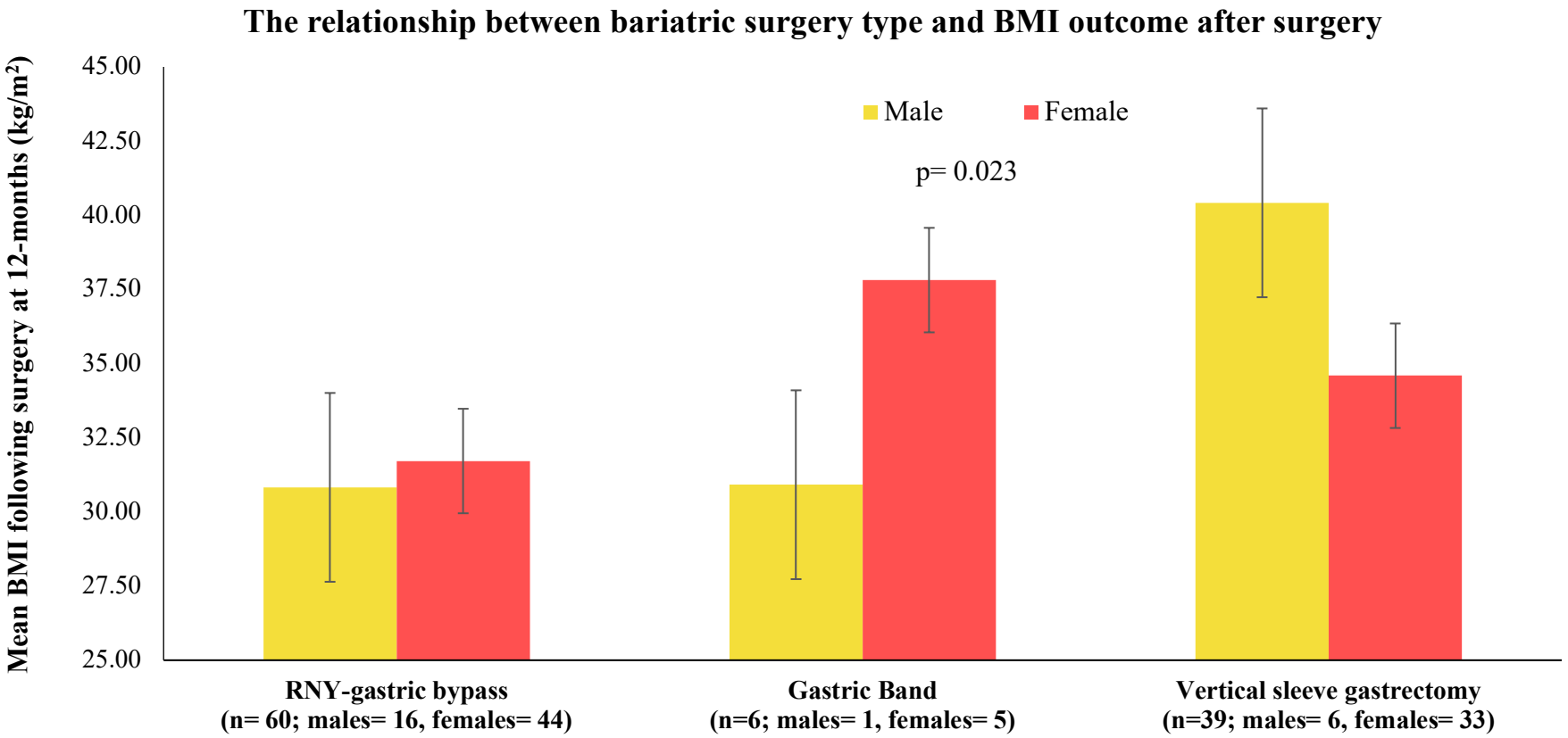

Figure 6.1 The mean BMI for post-surgery group who had undergone bariatric surgery and had available BMI outcome after 12-months of surgery (n=105). The blue and red bars represent males and females, respectively. Higher BMI outcome after 12-month surgery was seen among males compared to females in the gastric sleeve group (p= 0.021). The p-value shown was a significant association between BMI-post at 12 months and surgery types, adjusted for age, sex, ethnicity and T2DM status (after surgery).

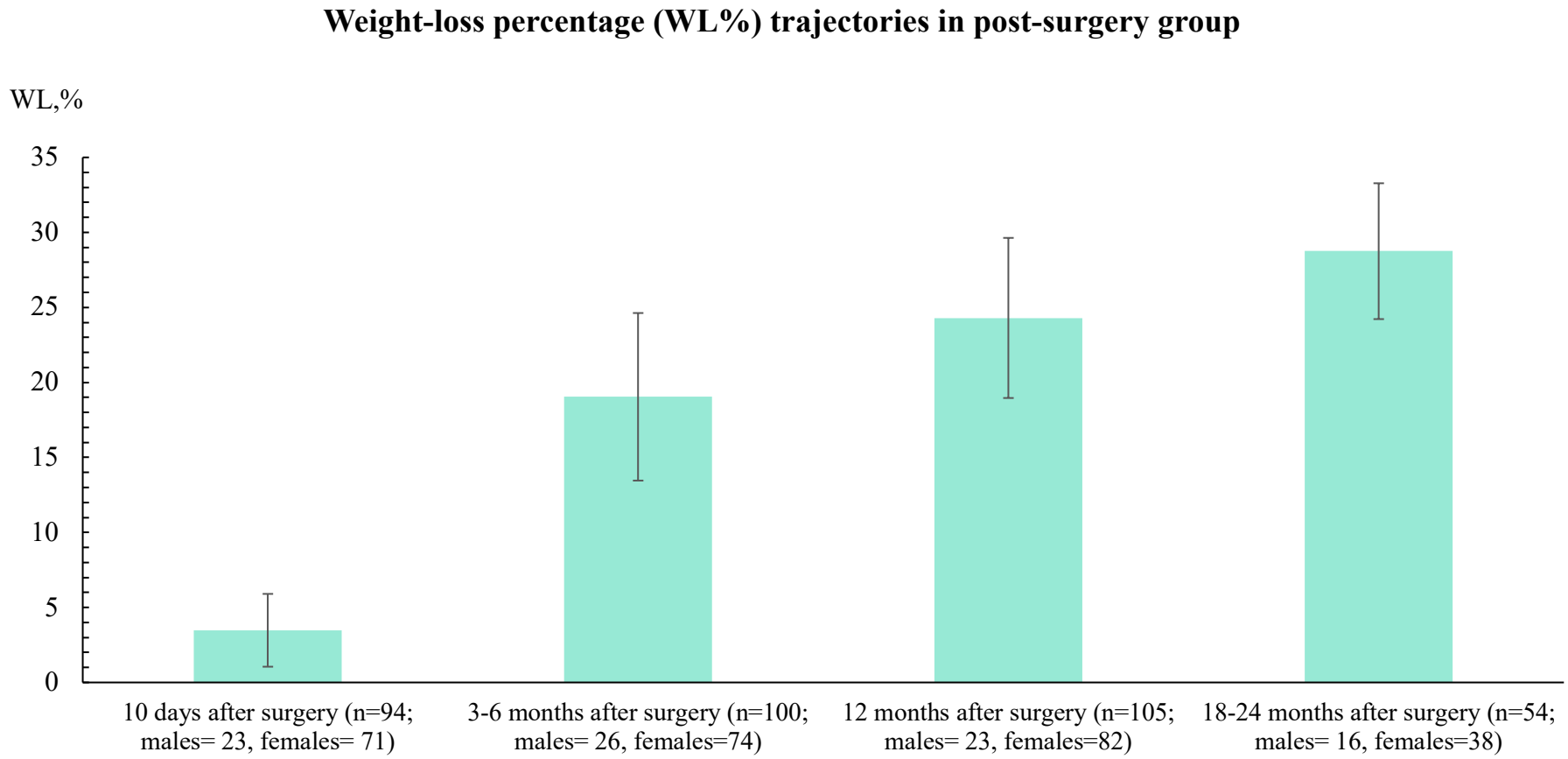

Figure 6.2 The weight-loss percentage for post-surgery group who had undergone bariatric surgery and had available weight-loss percentage outcome in current study.

6.3.5 Longitudinal dataset analysis

In the longitudinal analysis dataset (n=20), the majority of participants were female (80%, n=16) and the overall mean age was 50 (±11.24) years. The average time between TAS-20 questionnaires answered before and after surgery was 10.79 (±2.75) months.

The mean BMI for Group 3 participants was 44.0 (±8.56) kg/m^2, compared to 47.27 (±7.15) kg/m^2 before surgery and no significant mean BMI difference was observed ($p>0.05$). The mean %WL was 8.27%, 27.88% and 38.73% at 10 days (visit 4), six months (visit 5), and 12 months (visit 6) after surgery, respectively. There was a significant difference between T2DM prevalence before and after surgery ($\chi_{(2)}= 7.60$, $p = 0.022$): this is an expected outcome.

6.3.5.1 Cross-sectional analysis of TAS-20 and HADS scores

Due to lack of power in the longitudinal dataset, Pearson's analyses must be regarded as preliminary.

Moderate negative correlations were seen between baseline BMI and total TAS-20 scores ($r_{(20)}=-0.51$, $p=0.023$). The EOT subscale showed the highest negative correlation ($r_{(20)}=-0.53$, $p=0.016$) with baseline BMI when tested before surgery. Analysis by sex shows that females with lower baseline BMI scored higher in the total TAS-20 score ($r_{(16)}=-0.60$, $p=0.014$), DIF ($r_{(16)}=-0.51$, $p=0.043$) and EOT ($r_{(16)}=-0.66$, $p=0.005$).

In data obtained before-surgery, the TAS-20-DIF subscale was significantly correlated with the HADS-Depression ($r_{(19)}=0.55$, $p= 0.016$) and HADS-Anxiety ($r_{(19)}=0.48$, $p= 0.037$). There was also a significant correlation between the total TAS-20 score and the HADS-Depression subscale before surgery ($r_{(19)}=0.55$, $p= 0.016$).

In cross-sectional analysis of data obtained after the same participants had surgery, the TAS-20-DIF subscale was significantly correlated with HADS-Depression ($r_{(13)}$=0.77, p= 0.002) and HADS-Anxiety scores ($r_{(14)}$=0.75, p= 0.002). No correlations were seen between the EOT subscale and other psychological constructs when tested before or after surgery.

6.3.5.2 Longitudinal analysis: how did surgery affect TAS-20 scores in each individual and *vice versa*?

Figure 6.3 shows the change in TAS-20 scores for each participant. The total TAS-20 scores from the same individuals before and after surgery were significantly correlated ($r_{(20)}$=0.54, p= 0.015). The mean pre-surgery total TAS-20 score was nominally higher (51.10±9.48) than the mean post-surgery total TAS-20 score (49.05±11.50). However, a paired t-test showed that the difference in mean TAS-20 scores before and after surgery in Group 3 was not statistically significant (p> 0.05).

Pre-surgery total TAS-20 score was negatively correlated with %WL six months after surgery ($r_{(8)}$=-0.74, p= 0.037) and the pre-surgery EOT subscale score ($r_{(8)}$=-0.78, p= 0.022). There was also a very high negative correlation between %WL 12 months after surgery and the pre-surgery DDF subscale score ($r_{(3)}$=-0.998, p= 0.044).

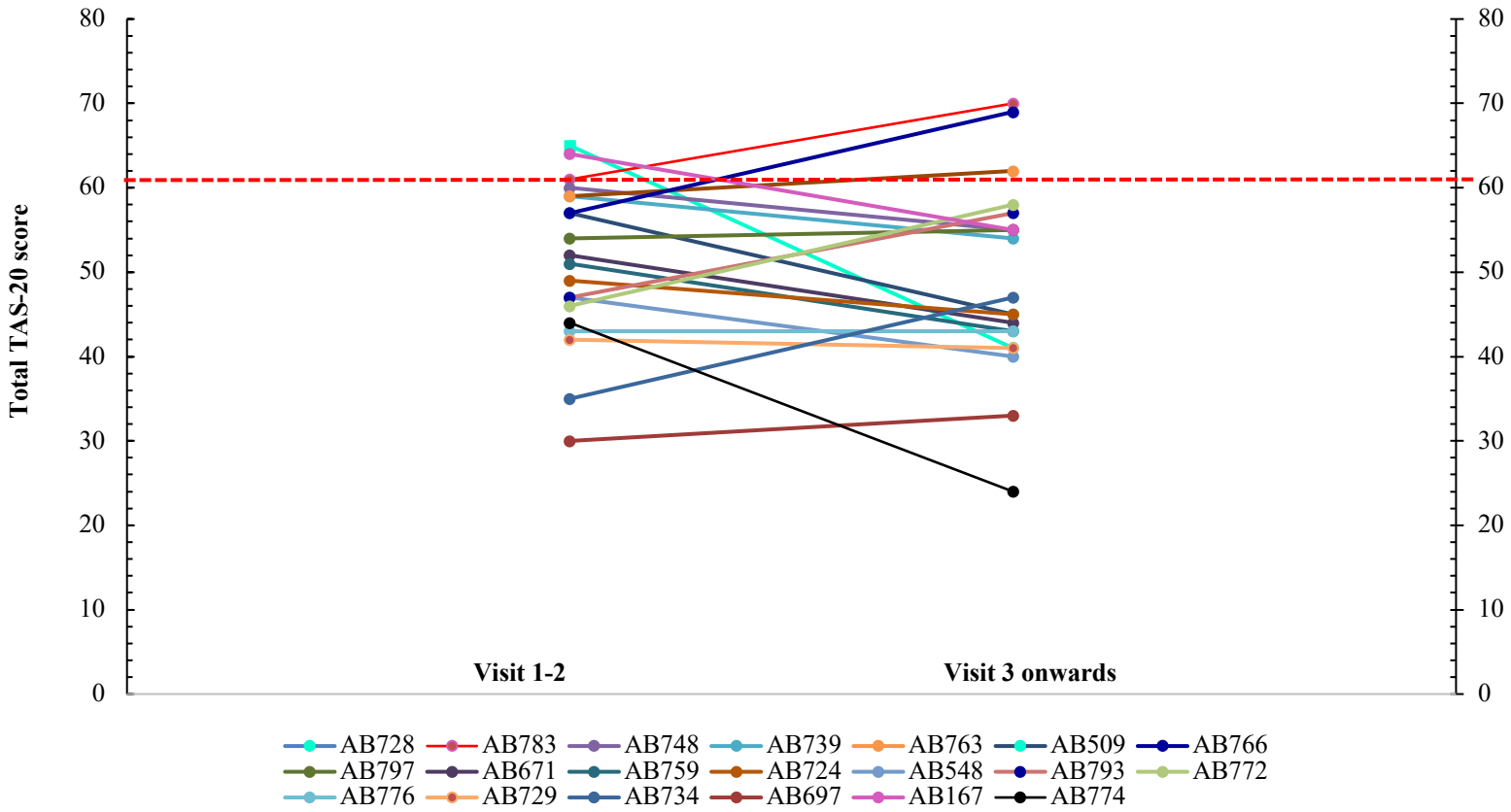

Figure 6.3 The change in TAS-20 score within the same individuals at different follow up visits. The average time between tests (follow-ups) was 10.79 (±2.75) months. The red line is the clinical threshold for alexithymia (Total TAS-20 score ≥61).

6.4 DISCUSSION

The current study was conducted to investigate the implications of TAS-20 scores and various psychological measures in a clinical cohort of severely obese adults (PMMO cohort). The TAS-20 questionnaire is designed to investigate alexithymia as a multi-faceted construct, so we also examined associations between TAS-20 subscales (DDF and DIF: difficulty in describing and identifying feelings/emotions, and EOT: externally oriented thinking) and psychological measures of depression and anxiety.

To date, the question whether alexithymia and depression are distinct/independent constructs or whether they overlap/co-exist is still unresolved, although the prevalence rates and associations with alexithymia and/or depression are well-documented in obesity and eating disorders. In previous chapters (Chapter 3 and Chapter 4), we saw both psychological constructs: alexithymia and depression associated with BMI and obesity in a general population (NFBC1966). In the adult Finnish population, only a fraction of the affected people continued over time to be in the alexithymic category, which could indicate that alexithymia is a transitory condition, at least in some individuals. As described in Chapter 4 of this thesis, alexithymia and depression have a significant impact on obesity among adults in the general Northern Finnish population which suggests that alexithymia and depression co-exist and are linked to BMI. Further investigation into this relationship in severely obese cohort may, thus, provide validation of our findings in general population.

Participants who had alexithymia when tested before surgery, had higher current depressive and anxiety symptoms. Similarly, participants who had alexithymia when tested after they had surgery, also reported higher depressive symptoms. Mental health is essential in determining healthy well-being and it is important to recognise alexithymia and depression as they may have a negative impact on the outcome of various disorders and on treatment relationships.

In contrast with epidemiological studies that reported no associations between obesity and depression among men(146, 147, 154, 368), this study found that there was an association between depressive symptoms (HADS) and alexithymia measures (DIF and DDF subscales) among obese males and females either pre- or post-surgery. In the general population (NFBC1966) studied in Chapter 4 of this thesis, we also found that, at the age of 46, males with a history of depression scored higher in the DIF subscale than males with no history of depression. This finding could be explained by the significant correlations between the subscales of depressive symptoms and TAS-20 in both PMMO and NFBC1966 cohorts.

This study was able to replicate alexithymia prevalences that have been reported in other obesity cohorts (369, 370). Similarly, to our findings on the TAS-20 subscales in the general population, it is DIF and DDF that are related to depression (but not the EOT subscale): we also found in the longitudinal subset of the PMMO cohort that there was no association between EOT and other psychological measures. These results are in agreement with previous reports showing that DIF was correlated with emotional eating (371), addiction (191), and that higher DIF scores were found in women with morbid obesity and emotional eating (43).

In Chapter 3 of this thesis, I found that the TAS-20 mean differences against BMI groups (WHO classification) and BMI transition groups was associated with the EOT subscale. In that general population, obese males and females had higher EOT subscale scores than individuals in the normal BMI group. Also, individuals with lower scores on the EOT subscale lost more weight/gained less weight than those with higher scores on that subscale. Based on the preliminary findings in this severely obese cohort, we can suggest that there is a relationship between alexithymia, depression and obesity. However, the directions of causality between those traits yet to be determined.

There were significant associations between %WL and the pre-surgery total TAS-20 score (and its subscales; DDF, DIF and EOT) in the longitudinal subset. However, the longitudinal results must be considered in the light of the limited sample size (n=20) and the non-randomised nature of sample selection in the pre- and post-surgery groups. We also had no matched controls for the pre- and post-surgery groups.

The strengths of the present study include the cross-sectional and longitudinal designs, in clinically-morbidly obese patients, which further strengthens the theoretical models of emotional dysregulation and obesity and complements work in general population cohorts well.

Since current findings were obtained in the niche area of morbid obesity, the heterogeneity of reported clinical binge-eating disorder and depression diagnosis among the PMMO participants could not be avoided. This was due to limited clinical data resources from different recruitment sites and may also have been affected by the desire among PMMO participants (pre-surgery group) to present themselves more positively, in an attempt to be found suitable for bariatric surgery.

Overall, the results here can be informative as psychological predictors of bariatric surgery outcomes. These findings raise intriguing questions regarding the nature and extent of the mechanism linking emotional processing and obesity outcomes. This study provides additional evidence that mental health conditions are important in obese individuals who are trying to lose their weight through surgery. Therefore, weight-loss interventions should include strategies that can improve mental health outcomes among obese individuals. Healthy coping modules addressing relevant emotional factors could be beneficial to bariatric surgery patients.

CHAPTER 7 General Discussion and Conclusions

7.1 Introduction

In this research study, I sought to determine the applicability of emotional processing deficits; alexithymia to aid in understanding obesity. The aim was to investigate patterns of alexithymia and depression in relation to obesity in an adult general population and in a clinical cohort of obese patients. It was anticipated that the findings of the study could inform clinical obesity management as well as indicate further areas of research. I used pre-existing self-report measures of alexithymia and depressive symptoms to investigate the associations between these constructs and obesity. Additionally, a genome-wide association study was conducted, which explored the role of genetics in alexithymia. It should be noted that I did not undertake formal causal analyses for example using Mendelian randomisation approaches, which were not possible due to the lack of instruments for alexithymia or structural causal modelling (372), but simply conducted association analyses which included both cross-sectional and longitudinal models over up to 15 years' time period.

Here, I will first discuss the findings from the study with reference to alexithymia and its subscales, demonstrating how the findings relate to existing literature and identifying new knowledge which may inform future clinical practice. Second, the implications of alexithymia and depression as a conceptual framework for obesity in light of existing models will be discussed. Further consideration of the implications of the findings with reference to current clinical practice and for future research is presented in the conclusion of this chapter.

7.2 The relationship between alexithymia and obesity

The aim of the study was to investigate whether there is a relationship between alexithymia and adiposity measures in a longitudinal cohort of the Northern Finnish population, and severely-obese patients seeking surgical weight-loss interventions. The results established that there was a clear relationship between alexithymia and obesity:

1) TAS-20 score is significantly associated with BMI and WHR in both adult and adolescent populations considered (NFBC1966 and NFBC1986).
2) Moreover, the prevalence of alexithymia is higher in severely-obese adults seeking bariatric surgery in the UK (PMMO clinical trial), than in general populations which confirms previous findings of high levels of alexithymia in people with obesity (22, 23, 60, 243, 373).
3) In a small subset of PMMO participants tested both before and after surgery (n=20, longitudinal subgroup), percentage weight-loss (%WL) was associated with pre-surgery TAS-20 score. Participants with higher %WL scored lower total TAS-20.
4) Alexithymia at age 31 years predicted BMI at age 46 years in the general population (NFBC1966 longitudinal data). Based on BMI predictive models over the 15-year study period, either continuous TAS-20 total score or alexithymia status had similar association with the total variance of BMI at later age (46 years), when adjusted for sex and socio-economic factors.
5) Correlational analysis on the TAS-20 scores revealed a significant positive association such that those participants with clinically-relevant alexithymia (TAS-20≥ 61) had higher mean BMI and WHR either at the ages of 16, 31 and 46 years.
6) Participants with stable TAS-20 scores also had more stable BMIs.
7) In our study, adolescent NFBC1986 participants with alexithymia had higher BMI compared to non-alexithymic participants. This is in accordance with a recent adolescent obesity study that showed that emotion regulation difficulties mediate the relationship between mindfulness abilities and emotional eating (374).

7.2.1 TAS-20 subscales and obesity

The overall relationships described above were reflected in particular subscales of the TAS-20 score. In the longitudinal part of the study, NFBC1966 participants who had the greatest decrease or increase in BMI had higher mean TAS-20 scores (and DIF and EOT subscale), and, those with stable TAS-20 scores also had more stable BMIs. In addition, DIF and EOT subscales are independently predictive of BMI in the NFBC1966 study, consistent with a recent study by Di Monte *et al.* (2020) which found that the DIF subscale of TAS-20 predicted weight for obese patients seeking surgical interventions (110). Women with obesity also scored higher on the DIF subscale compared to women with normal BMI (23, 60).

In addition, our data indicate that the observed changes in BMI over the 15-year period were related to changes in the EOT subscale. Individuals with higher scores on the EOT subscale gained more weight than those with lower scores on that subscale. This is in line with an earlier study (370) that found individuals with higher BMI (>26.4 kg/m^2) are more likely to score higher on the EOT subscale, which reflects the hypothesis of *la pensée operatoire* (375) or operative thinking in alexithymia. The hypothesis of operative thinking by Marty and de M'Uzaan (1963) which refers to psychologically-ill patients with alexithymia perceived themselves and others as objects rather than subjects. In other words, they tended to ignore their own thoughts and feelings and they failed to create mental representation as thinking, feeling and experiencing as themselves and others (108). The authors suggest that such impairment in cognitive style of emotional processing is consistent with alexithymia diagnostic criteria. In addition, the findings from our study are consistent with Gross's model (4) on the crucial role of emotional functioning in determining the relative health-benefit.

Sundararajan and Schubert (2005) explain alexithymia and related affective disorders within the framework of Semiotic theory of Charles Sanders Peirce, whose core conceptual

implication is that “reflexive undertow” will determine thought processes that are reactive and representational. The authors propose using a taxonomy of verbal expression of self and emotions in order to understand the lack of introspection aspect of alexithymia (376). In addition, a recent review by Hobson *et al.* (2019) has proposed the alexithymia language hypothesis which suggests that language deficits contribute to increased alexithymia under the ‘multiple routes to alexithymia’ model (377). In line with this, contemporary theories suggest that alexithymia may be linked to general failure of interoception (14, 17, 378, 379). Early alexithymia theoretical models implied deficits in interoception both at subjective and objective levels (50). People with obesity also appear to present with deficits in interoceptive ability (203, 380-382), as well as deficits in emotion processing (383, 384). Efficient interoceptive abilities are essential to regulate both food intake and emotions. Both emotional and interoceptive ability ensure identification of hunger and satiety and provide accurate information about emotional states to prevent confusion between inner sensations and emotional processing.

Hypothalamic signalling is the best-studied aspect of the central control of feeding and energy expenditure. In particular, ghrelin and leptin are recognised as important endocrine energy-signalling molecules that travel from the periphery to the brain via the circulatory system, cross the blood brain barrier, and bind to receptors in the hypothalamic signalling pathway (203). Of note, the gut and brain axis (GBA) is also associated with brain health. Neuropeptides such neuropeptide Y (NPY) and cholecystokinin (CCK) have regulatory roles in GBA pathway and have been reported to regulate emotion, as well as food intake, and obesity (141), highlighting the psychobiological relationship between emotion processing and obesity. However, it is still unknown how the hypothalamic nuclei are being regulated by and connected to superordinate brain centres, including the reward and sensory system (136). Given that the concept of alexithymia shares common theoretical foundations with both deficits in interoceptive

sensibility and emotion regulation (127, 139), it is important to detect alexithymia and defects in interoception especially in patients with obesity who respond poorly to treatments, especially to mindfulness and cognitive-behavioural techniques.

7.3 The relationship between alexithymia, depression and obesity

Although many studies of obesity have focused on emotional processing deficits (alexithymia) and regulation of emotions (43, 369, 370, 373, 385), it is important to know whether depression also contributed to the relationship between alexithymia and obesity, because this might indicate potential avenues for prevention and intervention in obesity. In Chapter 4, I investigate the overall relationship between alexithymia, depression and obesity in the NFBC1966 cohort. I found an interaction between alexithymia, obesity group and sex on the 15-item Hopkins Symptoms Checklist (HSCL-13) score (current depressive symptoms scale), and that alexithymia and depression are associated with obesity over the 15-year time period.

7.3.1 TAS-20 subscales and depression

DIF and DDF subscales (but not the EOT subscale) are associated with depression. This is consistent with the meta-analysis by Li *et al.* (2015), which concluded that the total TAS-20 scores, DIF and DDF, but not the EOT subscale, are related to depression (292), due to rumination (persistent and recyclic negative thinking) that presents among depressed patients (293, 294). This may be explained by the fact that DIF and DDF subscales are known as affective processing and TAS-20 questionnaire items of the DIF and DDF scales are mainly negative in their affective value and describe deficits and problems (e.g. “I don’t know what is going on inside me” or “It is difficult for me to find the right words for my feelings”).

In addition, depression may directly contribute to difficulties with identifying and expressing emotions in eating disorders (ED) patients (binge-eating disorder, anorexia and bulimia

nervosa) (26, 386, 387). ED patients with bingeing and purging behaviours may have increased impulsivity and emotional processing deficits (365, 366) which might imply underlying mechanisms linking alexithymia and depression. Previous studies have shown that there was an association between alexithymia and morbid obesity which supports our suggestion that alexithymia plays a role in further weight gain in obese individuals (43, 60, 61, 385).

In a predictive model for BMI, that includes TAS-20 score and HSCL-13 score as independent variables (predictors), adjusted for standard covariates, the TAS-20 total score but not HSCL score at baseline (31-year time point) predicted the BMI at the later age (46-year timepoint). This finding indicates that alexithymia, rather than depressive symptoms, independently associated with BMI. Since TAS-20 score is able to predict BMI over the 15 years in the NFBC1966, I constructed a depression predictive model to test whether we can predict future depressive symptoms. Male sex and alexithymia status (TAS-20 ≥61) at age of 31 years remained as significant risk factors for depressive symptoms at later age of 46 years which suggests complex relationships between these two. In addition, the longitudinal analysis of change in HSCL-13 revealed that males tend to have increased depressive symptoms (HSCL-13 score) at the later age compared to females. This is in line with previous studies that have shown that depressive symptoms follow a U-shaped curve over age (388-390). A recent study by Abrams and Mehta (2019), however, reported higher levels of depressive symptoms in midlife (ages 51-65) compared to late-life (ages above 65 years) in a longitudinal survey of the United States men and women aged 51 and older. The authors suggested that the ability or willingness to report feelings of depressed mood relative to somatic complaints are higher in males compared to females in the oldest age group (391).

In our study, males with either current depressive symptoms, or a history of clinical depression, had higher total TAS-20 score than females in the same depression groups, both at the age of

31 and at 46 years. This result indicates that sex/gender has an important effect for both alexithymia and depression psychological constructs; men tend to have lower experience and expression of anxiety and sadness, although the mechanisms underlying this are unclear (392).

7.3.2 Alexithymia and depression in the context of obesity

In Chapter 6, I investigate alexithymia and related-psychological measures in severely-obese adults from the PMMO clinical study. Severely obese patients with alexithymia (TAS-20≥ 61) had higher anxiety and depressive symptoms (measured by Hospital Anxiety Depression Scale; HADS) at baseline. Similarly, patients who had alexithymia when tested after surgery, also reported increased depressive symptoms (higher HADS score). BMI following surgery was positively associated with depression in a group of participants who were tested after surgery. In the NFBC1966 study, we found that males and females with obesity reported more current depressive symptoms (had higher mean HSCL-13 score) than those who were in the normal BMI group. These findings further confirm that alexithymia and depression are co-morbid/ co-exist in obesity. Thus, we can suggest that alexithymia and depression may have impact on the outcomes of weight loss treatment (before or after). The main findings from this thesis could fill in the research gap between psychology and obesity clinical practise.

There are continuing debates on the directions of causality (either obesity causing depression or depression causing obesity, or a third factor influencing both), previous epidemiological studies across different populations have provided evidence supporting relationships in both directions (393). The appraisal theory by Lazarus in the 1990s suggested that depression resulted from difficulties in emotion-cognition domains. Genetic variations and biological pathways may play a role in the inter-relationship between emotional processing and obesity as proposed in the conceptual model in this study (see Figure 1.4). However, the robust studies to systematically explore the causal mechanisms between obesity and psychological factors are

still lacking. In collaboration with the Finnish team, we conducted the first GWAS on TAS-20 to determine if there is a stable proxy for emotional processing difficulties the in general population. Separately, our data will form part of a GWAS meta-analysis of TAS-20 score. Currently there is a total 11802 of European ancestry-based participants from six different cohorts (including the NFBC1966) in the meta-analysis consortium and we aim to add more cohorts in the study. The outcome of this study will further our understanding of the genetic component in alexithymia, as well as hopefully elucidating biological mechanisms, and providing tools for causal Mendelian Randomisation studies.

7.4 TAS-20 score and HSCL-13 score GWAS findings in NFBCs

The genetic composition of alexithymia is largely unknown despite heritability evidence from family and twin studies (10-12, 272, 394, 395). Heritability is estimated to be 30-33% for alexithymia (10-12) and for depression heritability ranges between 25 and 45%(396-398). Despite the clear findings that point to neurobiological and genetic factors in the pathophysiology of alexithymia and depression, the genetic susceptibility for both constructs are largely unknown. In Chapter 5, I seek to identify common genetic variants or single nucleotide polymorphisms (SNPs) associated with TAS-20 and HSCL-13 scores, using cleaned genotype data of the two NFBCs. In a statistically-underpowered genome-wide association study (GWAS) dataset for TAS-20 score, undertaken training purposes, I found near-genome-wide significance association with an intronic variant of a gene associated with Parkinson's disease: *SNCAIP* (synuclein alpha interacting protein) in NFBC1966. Since alexithymia has been recognised as a prominent characteristic of Parkinson's disease and the *SNCAIP* gene has been associated with an increased risk of Parkinson's disease (343-345), we conclude that genetic variation within *SNCAIP*, may increase the risk of alexithymia. However, this will need larger scale meta-analyses and replication that is ongoing.

In addition, there were significant positive and negative associations ($p= 9 \times 10^{-7}$) between the top significant SNPs (rs2076562) and TAS-20 score in males and females, respectively. rs2076562 is located within the gene *SLC5A10* (solute carrier family 5) and four SNPs located within *MAP4K4* (Mitogen-Activated Protein Kinase 4) show gene × sex interaction influencing TAS-20 score in NFBC1966. This preliminary finding may suggest that *SLC5A10* and *MAP4K4* gene variants are implicated in interaction between alexithymia and sex but need to be confirmed again in larger studies.

Considering the polygenic features of alexithymia and depression constructs, the investigation on interactions between genes and/or environmental factors might represent a promising approach in genetic studies of alexithymia and depression. GWAS have been increasingly applied during the past decade in order to investigate genetic loci associated with various complex traits. In a meta-analysis of GWAS conducted by the Psychiatric Genomics Consortium (PGC), none of the investigated polymorphisms reached a genome-wide significance level, demonstrating no consistent association with depression (314). We also did not find suggestive genome wide significant genetic variations in our GWAS using HSCL-13 score data at age of 31 years in the NFBC1966. According to the review of Dunn et *al.* (399), the only genome-wide significant association with depression is rs1545843 *SLC6A15* and the variant is successfully replicated at a nominally significant level in four studies (400). Identifying genetic loci for depression is challenging because of the nature of the disease/disorder which is likely composed of subtypes with differences in biological aetiology and a heterogeneous genetic architecture. Recently genetic studies of alexithymia were performed by Mezzavilla and colleagues using an exome chip (covering functional genetic variants) in 585 healthy subjects (311) identified polymorphisms in *ABCB4*, *TP53AIP1, ARHGAP32* and *TMEM88B* genes. These associations however have not been replicated in independent samples and the genetic analyses performed so far were limited by small sample

sizes or lack of replication. Thus, there was a possibility of publication bias inherent in those genetic variants' studies. Additional caveat in the studies is the difficulty in measurement and diagnostics of depression.

Due to the lack of power and smaller effect on the regression analysis, GWAS for BMI in relation to TAS-20 and HSCL-13 scores was not performed in our study. A larger dataset would be ideal to investigate the interplay role of genetic variation in relation to alexithymia, depression and obesity. Polygenic risk profiling/scoring (PRS) could have been used in this study to summarise the genetic risk alleles and their corresponding effect sizes per individual (359, 401), however, it was thought to be out of scope for this study. In addition, Mendelian randomisation which uses genetic markers associated with a trait (derived from GWAS) as instrumental variables could be used to explore causality relationships between alexithymia, depression and obesity. Future studies to explore the directions of causality in the relationship between obesity, alexithymia and depression should benefit from these approaches which I will discuss further in later section. To date, more than 200 obesity-associated genetic variants have been discovered by GWAS: the greatest insights from GWAS of BMI is that the majority of discovered genes mostly actin the brain (402). Since biological pathways are likely to control feeding and weight gain, the homeostatic theory of obesity by David Marks (2015) and neurobehavioural disorder by Stephen O'Rahilly and Sadaf Farooqi (2008) should be considered in developing a consensus framework on human body weight homeostasis (403) in order to understand underlying mechanism of the obesity pandemic.

7.5 Theoretical Interpretations and Impacts

The results of previous studies on the relationships between alexithymia, and obesity showed that the prevalences of alexithymia and depressive symptoms are both higher in obese subjects. Depressive symptoms and alexithymia may also be linked to emotional eating in binge-eating

disorder (BED) (22, 23, 60, 61, 243, 373). In a recent Swedish diabetes clinical study, Melin *et al.* 2017 reported a significant increase in DIF scores among obese patients. Additionally, in males, the DIF subscale was strongly associated with abdominal obesity (404). Our study has also shown that males that have alexithymia have increased risk of mood disorders and have more difficulty in recognising/identifying/distinguishing emotions. Previous evidence supported our findings that the TAS-20 score and two of its subscales (DIF and EOT) are strongly associated with, and have a predictive effect on, BMI over a 15-year time period.

Affective disorders such as anxiety and depression are strongly associated with alexithymia and obesity (61, 405-408). Despite this, there is a lack of targeted treatment strategies for depressed, obese individuals: indeed, patients with psychological problems have commonly been screened out from weight-loss interventions since the 1950s. In current study, following psychological screening in Imperial Weight Centre, we found that morbidly obese patients with alexithymia had higher self-rated scores in depressive and anxiety domains at baseline.

7.6 Strengths of this thesis

This work benefits from analysis of both large-scale unselected population-based data sets and a clinical cohort with extreme phenotype, both richly phenotyped and with anthropomentric, clinical and psychological data available for the analyses.

The cross-sectional and longitudinal designs of both general and clinical cohort analyses add strength to our present study, which further strengthens the proposed conceptual model of alexithymia, depression and obesity. Thus, there is a potential clinical benefit from these findings.

7.7 Limitations and Future work

The inter-variability of self-reported measures (and the complexity of obesity and the psychological constructs investigated) could be a limitation in the interpretation of the psychological results. The statistical power gained by large numbers, and by use of a pre-existing dataset, is partially offset by the inability to carry out bespoke gold-standard interventional analyses (such as directly measuring *ad libitum* food intake in participants with different TAS-20 scores or assessing their interoception ability).

Additionally, as the current clinical cohort was conducted in a niche area of a morbidly obese population, there will be a heterogeneity of psychological profiles among them. Nearly half of individuals seeking treatment for severe obesity were reported to have at least one psychiatric disorder (409). The absence of matched controls in the PMMO clinical trial and limited clinical data resources obtained from different recruitment sites should also be noted as limitations in this study.

Genetics play an integral part in virtually all human diseases. As alexithymia has been associated with various mental and health problems, it is an intricate task to examine to which degree alexithymia is an independent construct. Hence, alexithymia represents a potential risk factor to consider for any psychological medical interventions.

Lifestyle and environmental exposures may also confound the relationships between obesity, depression and alexithymia that we observed in this study. Interoception has been associated with alexithymia (14), and failure to perceive and correctly interpret bodily sensations may also be an important mediating factor in the relationship between alexithymia and obesity. However, our current dataset does not allow exploration of this matter.

In the future, study of alexithymia and depression in relation to obesity should include consideration of analysis by sex in the analytical framework. Also alexithymia should be

considered in mental health or medical interventions. Future research on alexithymia and obesity should explore both genetic and phenotypic data on depression and eating behaviour to determine the causal mechanisms and characterise the interplay between them. Here, I propose in the following sections (7.7.1-5) a number of future investigations to address these matters.

7.7.1 Behavioural experimental study on the relationship between alexithymia and eating behaviour

An interesting extension of the findings described in this thesis might be a future direct observational study in which participants of known TAS-20 score were given *ad-libitum* access to food. This could directly confirm that statistical observations reported in this thesis.

Similarly, a direct investigation of interoceptive ability and eating behaviour (*ad-libitum* food intake) might be carried out, in the presence of positive, neutral or negative stimuli (designed to induce fear, pleasure, boredom, etc). Individuals with alexithymia and interoception deficits might be expected to eat more compared to others under all (positive/negative) circumstances. Further, it might be possible for skilled practitioners to design this intervention study to explore mechanisms: determining whether overeating is due to impaired interoception blunting satiety signals, or whether overeating in people with alexithymia primarily a maladaptive emotion-regulation strategy. The concept would be that if impaired interoception simply means they have lack of awareness for satiety signals, then perhaps people with high alexithymia scores will eat the same amount without any difference between stimulation conditions. If, however, the overeating represents a maladaptive emotion-regulation strategy, then people with alexithymia might eat more under stressful or upsetting situations, and/or they might choose more "comfort foods". Clearly, such an experiment would need careful design by psychologists and nutritionists to ensure validity.

If it were possible to design such an experiment and apply it to a group of people seeking to lose weight, it might lay the foundations for design of bespoke targeted therapeutic interventions to address their specific problem.

7.7.2 SNPs and exome variant look-up in NFBCs and PMMO genetic data

The heritability estimates for the TAS-20 total score are 30-33% according to previous alexithymia twin studies(10-12, 70). Under an additive model, our TAS-20 GWAS had more than 80% power at $\alpha = 5\%$ level for linear regression model. Nonetheless, this is underpowered for a genome-wide study: ideally, such analyses would include hundreds of thousands of participants. The GWAS was included here for training purposes and because NFBCs were joining in a larger study on alexithymia. This large-scale consortium has already been gathered to carry out these analyses. Careful phenotyping is essential, and if the "Multiple routes to alexithymia" concept is correct it might be useful in future genetic work to be able to distinguish "trait" alexithymia (using individuals whose scores were relatively stable over long periods), from "state" alexithymia which might vary according to depression or other exposures (using people with greater difference in scores between the two time points). This will require larger numbers than were available during the preparation of this thesis.

The phenotypic effects of top hits list from TAS-20 GWAS could then be examined in detailed and matched with existing genotyping, and whole exome sequencing data from participants in the PMMO cohort. Genetic risk scores (GRSs) will be established based on the GWAS-top-hit SNPs. A custom genotyping chip is being designed by Professor Alex Blakemore's research team, primarily for application within the PMMO cohort and another obesity cohort from the Middle East. This chip will contain SNPs that have been previously associated with body weight, BMI, obesity, diabetes, eating behaviours and various psychological disorders (autism, alexithymia, mental retardation, etc.) as well as rare mutations potentially causative of

monogenic obesity and diabetes. Genetic risk scores from the NFBCs will be validated using this new chip in PMMO and a new Middle Eastern obesity cohort being collected in Abu Dhabi.

7.7.3 Polygenic risk score analysis, and Mendelian randomisation studies between alexithymia, depressive symptoms, and obesity

When a GWAS has been carried out, there are a number of follow-on analyses that can be carried out. In particular, the results of individual SNPs can be combined in risk scores that have greater predictive power for individuals and can also be used as tools to explore directions of causality. PRSice is software that calculates polygenic risk score (PRS) using estimated published GWAS. Since alexithymia and depression both associated with obesity in NFBCs and PMMO, the next step is to determine whether there is shared genetic aetiology between alexithymia and depression and to determine whether either a PRS for TAS-20 or for HSCL-13 can predict BMI in NFBCs. Multiple linear regression tests can be used to examine associations between the BMI, TAS-20-PRS, TAS-20 scores, and HSCL-13 scores (a proxy measurement for current depressive status).

7.7.4 Investigations on the relationship between TAS-20 scores, depressive symptoms and eating behaviour in NFBC1966 and PMMO.

Eating behaviours among obese individuals serve as a pathophysiologic feature in obesity treatment outcomes. Several findings suggest that human emotions are central to eating and disordered eating behaviour (140, 200, 410). Moreover, previous researchers have also reported the prevalence of emotional eating and its associations with higher BMI (411, 412). Macht (1999) reported that females tend to report emotional eating (413) and in our weight-loss intervention clinical trial, we have data on their eating behaviours. Three Factor Eating Questionnaire (TFEQ) data is available at age of 46 years in the NFBC1966 and could be used to conduct a GWAS for eating behaviour in NFBC1966 (and other studies available).

7.7.5 Investigation of the relationship between TAS-20 scores, depression and health-related lifestyle behaviour, and its implications for BMI in NFBCs

Environmental exposures (smoking, and drinking habit) of the mother and father during the pre-natal of NFBC1986 participants, eating behaviour and pattern (type of food, food and drinks pattern) at 16 years old (NFBC1986), 31 and 46 years old (NFBC1966), physical activities, smoking, and drinking habit in 16 years old (NFBC1986), 31 and 46 years old (NFBC1986) could be cleaned and curated from my current database. Methods as described in Chapter 3 and Chapter 4 of this thesis would be used to investigate the relationship between TAS-20 score, depression and health-related lifestyle behaviour and its implications for BMI in the two NFBCs.

7.8 Conclusions

This work presented in this thesis confirms the importance of emotional processing in relationship to obesity and current weight-loss interventions. Further studies, combining genetic, behavioural and psychological factors are warranted to refine our understanding of the aetiology of obesity.

The findings from this thesis could be implemented to refine strategies used to improve mental health outcomes among obese individuals. The evaluation of alexithymia should be included in addition to existing mindfulness strategies for weight management among severely obese patients. The development of 'personalised' interventions to support emotional processing and, thereby, improve mental health outcomes among obese individuals should increase the effectiveness and facilitate long-term durability of weight-loss interventions.

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