Nature and nurture:

Insights from genetic, environmental and epigenomic studies of late-life depression

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Abstract

Late-life depression (LLD) is a significant public health problem. It is one of the most common neuropsychiatric disorders in later life, and is associated with increased disability, morbidity and mortality. This thesis aims to obtain new insights into its pathophysiology by exploring the genetic, environmental and epigenetic influences on LLD in samples from the Older Australian Twins Study and the Sydney Memory and Ageing Study.

First, the relative contributions of genetic and environmental influences on LLD and its co-variation with anxiety and hypertension were investigated in twin pairs. It replicated previous findings that LLD is significantly heritable, and additionally demonstrated that LLD shares genetic influences with anxiety but not with hypertension. Next, a systematic review of genetic association studies of LLD revealed there was limited research with a general lack of replication. The meta-analysis identified three variants (APOE ε2/ε3/ε4, BDNF Val66Met and SLC6A4 5-HTTLPR) as significantly associated with LLD. Using the Australian cohorts, replication of LLD or major depressive disorder (MDD) candidate single-nucleotide polymorphisms (SNPs) was attempted, with no significant results. Furthermore, the effects of early-life trauma on LLD were examined, with childhood emotional abuse and exposure to Holocaust trauma significantly predicting LLD. Neither type of early-life trauma showed significant gene-environment interactions with LLD or MDD candidate SNPs. Finally, an epigenome-wide association study of LLD was conducted using blood DNA methylation from monozygotic twin pairs. It identified 69 differentially methylated probes, some of which are located near or in genes that have previously been implicated in neuropsychiatric or neurodegenerative disorders. The genes associated with the top-ranked probes appeared to be enriched for a range of developmental processes, including central nervous system development and neurogenesis.

This thesis provides several novel contributions to the literature by identifying some important genetic, environmental and epigenetic influences on LLD. Taken together, the findings highlight the potential role of neurodevelopment in the pathophysiology of LLD, which can have significant implications for the prevention and treatment of LLD. Research on the genetics and epigenetics of LLD is clearly still at a nascent stage, and more research is warranted to further our understanding.

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ABSTRACT

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epigenetics of LLD is clearly still at a nascent stage, and more research is warranted to further our understanding.

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TABLE OF CONTENTS

Abstract ii

Acknowledgements v

List of Tables xi

List of Figures xiv

List of Abbreviations and Symbols xv

List of Gene Symbols xviii

Publications, Presentations and Awards xxi

1 Introduction 1

1.1 Context 1

1.2 Rationale 4

1.3 Objective and aims of this thesis 5

1.4 Thesis outline 5

2 Background 7

2.1 Depression in later life 7

2.1.1 Diagnosis 7

2.1.2 Epidemiology 9

2.1.3 Age of onset 10

2.1.4 Prognosis 12

2.1.5 Medical and psychiatric comorbidities of late-life depression 13

2.2 Neurobiological correlates of late-life depression 23

2.2.1 Structural neuroimaging 23

2.2.2 Functional neuroimaging 27

2.2.3 Post-mortem studies 31

2.3 Risk factors for late-life depression 36

2.3.1 Genetic risk factors 36

2.3.2 Gene-environment interactions 38

2.3.3 Potential epigenetics mechanisms 38

2.3.4 Biological and psychosocial risk factors 39

2.4 Theories on the pathophysiology of late-life depression 40
2.4.1 The monoamine hypothesis 40
2.4.2 The vascular hypothesis 40
2.4.3 The glucocorticoid cascade hypothesis 42
2.4.4 The neurotrophic hypothesis 43
2.4.5 The inflammatory hypothesis 44
2.4.6 Potential role of the microbiota-gut-brain axis 46
2.4.7 Cross talk between multiple systems 47
2.5 Summary and gaps in the current literature 48
3 Methods 50
3.1 Study samples 50
3.1.1 The Older Australian Twins Study (OATS) 50
3.1.2 The Sydney Memory and Ageing Study (Sydney MAS) 52
3.2 Measures 56
3.2.1 Psychosocial measures 56
3.2.2 Biological measures 59
3.2.3 Covariates 60
3.3 Study designs and statistical methods 61
3.3.1 Statistical packages 61
3.3.2 Descriptive data analyses 61
3.3.3 Missing data 61
3.3.4 Heritability and genetic correlations 61
3.3.5 Systematic review and meta-analysis of genetic association studies 64
3.3.6 Genetic, environmental and gene-environment predictors 65
3.3.7 DNA methylation changes 66
4 Genetic and Environmental Influences on Late-Life Depression and Its Covariation with Associated Phenotypes 68
4.1 Introduction 68
4.2 Methods 73
4.2.1 Participants 73

4.2.2 Measures 74
4.2.3 Statistical analyses 74
4.3 Results 75
4.3.1 Phenotypic concordance 76
4.3.2 Multivariate genetic modelling 76
4.4 Discussion 78
5 Genetic Association Studies of Late-Life Depression 83
5.1 Introduction 83
5.2 Systematic review and meta-analysis 84
5.2.1 Methods 84
5.2.2 Results 86
5.3 Candidate polymorphisms 98
5.3.1 Methods 98
5.3.2 Results 99
5.4 Discussion 103
6 Impact of Early-Life Trauma on Late-Life Depression 108
6.1 Introduction 108
6.1.1 Childhood maltreatment 108
6.1.2 War-related trauma 109
6.1.3 Gene-environment interactions in depression 110
6.2 Methods 112
6.2.1 Participants 112
6.2.2 Measures 113
6.2.3 Testing for deviations from Hardy-Weinberg Equilibrium 114
6.2.4 Statistical analyses 114
6.3 Results 115
6.3.1 Prevalence of childhood maltreatment 115
6.3.2 Effects of childhood maltreatment and Holocaust trauma on late-life depression 115
6.3.3 Gene-environment interactions 116

6.4 Discussion 123
7 DNA Methylation in Late-Life Depression 128
7.1 Introduction 128
7.2 Methods 133
7.2.1 Participants 133
7.2.2 Measures 133
7.2.3 Procedures 133
7.2.4 Epigenome-wide association study 134
7.2.5 Pathway analysis 134
7.2.6 Blood-brain DNA methylation comparison 135
7.3 Results 135
7.3.1 Participants 135
7.3.2 Epigenome-wide association studies 136
7.3.3 Pathway analysis 141
7.3.4 Blood-brain DNA methylation correlations 142
7.4 Discussion 144
8 General Discussion and Conclusions 149
8.1 Review of thesis objective and aims 149
8.2 Summary of key findings 150
8.2.1 Genetic epidemiology of late-life depression and associated phenotypes 150
8.2.2 Susceptibility loci for late-life depression 151
8.2.3 Enduring effects of early-life trauma 151
8.2.4 DNA methylation signatures in late-life depression 153
8.3 The role of neurodevelopment in late-life depression 153
8.4 Clinical implications 155
8.4.1 Preventive strategies for late-life depression 156
8.4.2 Ethical challenges of predictive genetic or epigenetic testing 159
8.4.3 Therapeutic options for late-life depression 160
8.5 Methodological and conceptual considerations and limitations 161
8.5.1 Small sample sizes 161

8.5.2 Definition of depression....162
8.5.3 Heterogeneity in depression....162
8.5.4 Polygenic and multifactorial nature of late-life depression....164
8.5.5 Inherent imprecision in a descriptive nosology....164
8.6 Future avenues of research....166
8.7 Concluding remarks....167
References....169
Appendix A. Supplemental Tables....233
Appendix B. Additional Analyses For Chapters 5 and 6....239
Appendix C. Published Manuscript....252

LIST OF TABLES

Table 1.1. Leading causes of disability in populations aged 60 years and over, estimated for the year 2015 (World Health Organization, 2015). 3
Table 2.1. DSM-5 and ICD-10 diagnostic criteria for (major) depressive disorder/episode. 8
Table 2.2. Diagnostic criteria for metabolic syndrome (Alberti et al., 2009). 16
Table 2.3. Summary of clinicopathological studies of LLD. 33
Table 2.4. Clinical features associated with vascular versus non-vascular depression (Aizenstein et al., 2016). 42
Table 3.1. Demographics of twin pairs in OATS at baseline (*M* ± *SD* or *n* (%)). 51
Table 3.2. MAS sample demographics at baseline. 53
Table 3.3. Neuropsychological tests used to form domain scores in OATS and Sydney MAS. 55
Table 3.4. Classification of Childhood Trauma Questionnaire-Short Form subscale scores according to Bernstein & Fink (1998). 58
Table 4.1. Summary of published heritability studies of LLD or depressive symptoms. 70
Table 4.2. Characteristics of the sample used in this study (*M* ± *SD* or *n* (%)). 76
Table 4.3. Heritability estimates for LLD, anxiety and hypertension. 77
Table 4.4. Estimates of genetic and environmental correlations between LLD, anxiety and hypertension in older adult twins. 78
Table 5.1. Genes investigated in included studies and their functions. 89
Table 5.2. Results of meta-analyses of candidate gene studies of late-life depression. 90
Table 5.3. Summary of findings from included candidate gene studies not in meta-analyses. 94
Table 5.4. Demographic information of the Sydney MAS subsample included in this study (*M* ± *SD* or *n* (%)) 99
Table 5.5. Genotype frequencies for candidate polymorphisms and tests for deviations from Hardy-Weinberg equilibrium. 100
Table 5.6. Logistic regression of *APOE* ε2 carrier status in predicting late-life depression. 101
Table 5.7. Logistic regression of *APOE* ε4 carrier status in predicting late-life depression. 101
Table 5.8. Logistic regression of *BDNF* Val66Met in predicting late-life depression. 101
Table 5.9. Logistic regression of *GNB3* C825T in predicting late-life depression. 102

Table 5.10. Logistic regression of *MTHFR* C677T in predicting late-life depression. 102
Table 5.11. Logistic regression of rs40465 in predicting late-life depression. 102
Table 6.1. Sample demographics (*M* ± *SD* or *n* (%)) 113
Table 6.2. Genotype distributions and tests for deviation from Hardy-Weinberg equilibrium for *APOE* ε2/ε3/ε4 (rs429358 and rs7412), *BDNF* Val66Met, *GNB3* C825T, *MTHFR* C677T and rs40465. 114
Table 6.3. Prevalence of childhood maltreatment assessed using the CTQ-SF (*n* (%)) 115
Table 6.4. Logistic regression of childhood maltreatment in predicting late-life depression. 116
Table 6.5. Logistic regression of Holocaust trauma in predicting late-life depression. 116
Table 6.6. Logistic regression of the interaction between *APOE* ε2 allele and childhood maltreatment in predicting late-life depression. 117
Table 6.7. Logistic regression of the interaction between *APOE* ε2 allele and Holocaust trauma in predicting late-life depression. 117
Table 6.8. Logistic regression of the interaction between *APOE* ε4 allele and childhood maltreatment in predicting late-life depression. 118
Table 6.9. Logistic regression of the interaction between *APOE* ε4 allele and Holocaust trauma in predicting late-life depression. 118
Table 6.10. Logistic regression of the interaction between *BDNF* Val66Met genotype and childhood maltreatment in predicting late-life depression. 119
Table 6.11. Logistic regression of the interaction between *BDNF* Val66Met genotype and Holocaust trauma in predicting late-life depression. 119
Table 6.12. Logistic regression of the interaction between *GNB3* C825T genotype and childhood maltreatment in predicting late-life depression. 120
Table 6.13. Logistic regression of the interaction between *GNB3* C825T genotype and Holocaust trauma in predicting late life-depression. 120
Table 6.14. Logistic regression of the interaction between *MTHFR* C677T genotype and childhood maltreatment in predicting late-life depression. 121
Table 6.15. Logistic regression of the interaction between *MTHFR* C677T genotype and Holocaust trauma in predicting late-life depression. 121

Table 6.16. Logistic regression of the interaction between rs40465 and childhood maltreatment in predicting late-life depression.....122
Table 6.17. Logistic regression of the interaction between rs40465 and Holocaust trauma in predicting late-life depression.....122
Table 7.1. Summary of DNA methylation studies of depression (including only adult samples with no psychiatric comorbidity).....129
Table 7.2. Distribution of probe types before and after quality control.....134
Table 7.3. Sample demographics ($M \pm SD$ or n (%))....136
Table 7.4. Details of the significantly differentially methylated probes (n = 69) identified in the full twin sample EWAS.....138
Table 7.5. Top 10 enriched KEGG pathways.....141
Table 7.6. Significantly enriched GO biological processes.....142
Table 7.7. Correlations of DNA methylation levels in blood with four brain regions.....143

LIST OF FIGURES

Figure 3.1. OATS flowchart. 52

Figure 3.2. MAS flowchart. 54

Figure 3.3. Example of a univariate ACE model. A – additive genetic effects, C – shared environmental effects, E – unique environmental effects. The additive genetic correlation is 1.0 for MZ twins and 0.5 for DZ twins. 62

Figure 3.4. An example of a multivariate model of 3 traits using Cholesky decomposition. A – additive genetic effects, C – shared environmental effects, E – unique environmental effects. The additive genetic correlation is 1.0 for MZ twins and 0.5 for DZ twins. 63

Figure 4.1. Path model with standardised path estimates for the best-fitting model for LLD, anxiety, and hypertension in older adults. Significant paths are indicated in bold. 77

Figure 5.1. Study selection flowchart according to MOOSE guidelines (Stroup et al., 2000). 88

Figure 5.2. Forest plots of statistically significant meta-analyses. 91

Figure 5.3. Funnel plots of statistically significant meta-analyses. 97

Figure 7.1. Manhattan plot of unadjusted *p*-values of all probes tested in the EWAS using the full MZ twin sample. 137

LIST OF ABBREVIATIONS AND SYMBOLS

-2LL	minus two log-likelihood
27K	Illumina HumanMethylation 27K BeadChip
3' UTR	3' untranslated region
450K	Illumina HumanMethylation 450K BeadChip
5' UTR	5' untranslated region
5-HT	Serotonin
Aβ	beta-amyloid
ACTH	adrenocorticotropic hormone
AD	Alzheimer's disease
AGECAT	Automated Geriatric Examination for Computer Assisted Taxonomy
AIC	Akaike's Information Criteria
AVP	arginine-vasopressin
BD	Bipolar disorder
BDI	Beck Depression Inventory
BMI	body mass index
CAMDEX	Cambridge Mental Disorders of the Elderly Examination
CCMD-3	Chinese Classification of Mental Disorders, 3rd edition
CES-D	Centre for Epidemiologic Studies Depression Scale
CI	confidence interval
CIDI	Composite International Diagnostic Interview
CRH	corticotropin-releasing hormone
CRP	C-reactive protein
CTQ-SF	Childhood Trauma Questionnaire-Short Form
DDES	Duke Depression Evaluation Schedule
df	degrees of freedom
DNA	deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	diffusion tensor imaging

DZ	dizygotic
EEA	equal environment assumption
EOD	early-onset depression
EWAS	epigenome-wide association study/studies
FA	fractional anisotropy
GAS	Goldberg Anxiety Scale
GDS	Geriatric Depression Scale
GMS	Geriatric Mental State Examination
GO	Gene Ontology
GWAS	genome-wide association study/studies
GxE	gene-environment
HAM-D	Hamilton Rating Scale for Depression
HPA	hypothalamic-pituitary-adrenal
HR	hazard ratio
HWE	Hardy-Weinberg equilibrium
ICD	International Classification of Diseases
IL	interleukin
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC	locus coeruleus
LLD	late-life depression
LOD	late-onset depression
M	mean
MADRS	Montgomery-Åsberg Depression Rating Scale
MAPK	mitogen-activated protein kinase
MAS	Memory and Ageing Study
MCI	mild cognitive impairment
MDD	major depressive disorder
MDI	Major Depression Inventory
MHR	mortality hazard ratio
MINI	Mini International Neuropsychiatric Interview

MMSE	Mini-Mental State Examination
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MZ	monozygotic
n.s.	non-significant
NESB	non-English speaking background
NFT	neurofibrillary tangles
NIMH DIS	National Institute of Mental Health Diagnostic Interview Schedule
OATS	Older Australian Twins Study
OR	odds ratio
PET	positron emission tomography
PHQ	Patient Health Questionnaire
rCBF	regional cerebral blood flow
RR	relative risk
SCID	Structured Clinical Interview for DSM-IV Axis I Disorders
SD	standard deviation
SE	standard error
SMD	standardised mean difference
SNP	single-nucleotide polymorphism
SSAGA	Semi-Structured Assessment for the Genetics of Alcoholism
TNF	tumour necrosis factor
TSS1500	200-1500 bases upstream of transcriptional start site
TSS200	0-200 bases upstream of transcriptional start site
VaD	vascular dementia
WebGestalt	Web-based Gene Set Analysis Toolkit
WMHs	white matter hyperintensities
YLD	years of healthy life lost due to disability

LIST OF GENE SYMBOLS

ACE	angiotensin I converting enzyme
AGRN	agrin
AGTR1	angiotensin II receptor type 1
AKT1	AKT serine/threonine kinase 1
APOE	apolipoprotein E
ASPHD2	aspartate beta-hydroxylase domain containing 2
BAP1	BRCA1 associated protein 1
BDNF	brain-derived neurotrophic factor
C1QTNF8	C1q and TNF related 8
CASZ1	castor zinc finger 1
CCDC25	coiled-coil domain containing 25
CD160	CD160 molecule
CFDP1	craniofacial development protein 1
CHRNA2	cholinergic receptor nicotinic alpha 2 subunit
CNTF	ciliary neurotrophic factor
COMT	catechol-O-methyltransferase
CRP	C-reactive protein
CRY1	cryptochrome circadian regulator 1
CRY2	cryptochrome circadian regulator 2
C10orf90	chromosome 10 open reading frame 90
DIP2C	disco interacting protein 2 homolog C
DNER	delta/notch like EGF repeat containing
ELOVL2	ELOVL fatty acid elongase 2
EML4	echinoderm microtubule associated protein like 4
EN2	engrailed homeobox 2
ESCO2	establishment of sister chromatid cohesion N-acetyltransferase 2
FAM160A1	family with sequence similarity 160 member A1
FBLN7	fibulin 7

FGGY	FGGY carbohydrate kinase domain containing
FKBP5	FK506 binding protein 5
GNB3	G protein subunit beta 3
GPR31	G protein-coupled receptor 31
GRAMD4	GRAM domain containing 4
GTF2IRD1	GTF2I repeat domain containing 1
ICA1L	islet cell autoantigen 1 like
IL1B	interleukin 1 beta
IL10	interleukin 10
JSRP1	junctional sarcoplasmic reticulum protein 1
KLHL2	kelch like family member 2
LCLAT1	lysocardiolipin acyltransferase 1
LINC00336	long intergenic non-protein coding RNA 336
LINC00461	long intergenic non-protein coding RNA 461
LRP1	LDL receptor related protein 1
MBP	myelin basic protein
MNT	MAX network transcriptional repressor
MT1X	metallothionein 1X
MTHFR	methylenetetrahydrofolate reductase
NECTIN2	nectin cell adhesion molecule 2
NFIC	nuclear factor I C
NHLH2	nescient helix-loop-helix 2
NR3C1	nuclear receptor subfamily 3 group C member 1 (also known as *GR* – glucocorticoid receptor)
NR3C2	nuclear receptor subfamily 3 group C member 2 (also known as *MR* – mineralocorticoid receptor)
NTRK2	neurotrophic receptor tyrosine kinase 2
PCLO	piccolo presynaptic cytomatrix protein
PHYKPL	5-phosphohydroxy-L-lysine phospho-lyase
PIWIL1	piwi like RNA-mediated gene silencing 1

PLIN3	perilipin 3
PPARG	peroxisome proliferator activated receptor gamma
PRKAR1B	protein kinase cAMP-dependent type I regulatory subunit beta
RAB11FIP4	RAB11 family interacting protein 4
SGMS2	sphingomyelin synthase 2
SLC35F3	solute carrier family 35 member F3
SLC6A4	solute carrier family 6 member 4
SOHLH1	spermatogenesis and oogenesis specific basic helix-loop-helix 1
STX1B	syntaxin 1B
TAPBP	TAP binding protein
TBL2	transducin beta like 2
TEF	TEF, PAR bZIP transcription factor
TFCP2	transcription factor CP2
TMEM100	transmembrane protein 100
TENM2	teneurin transmembrane protein 2
TNF	tumour necrosis factor
TPH2	tryptophan hydroxylase 2
TROVE2	TROVE domain family member 2
TSNARE1	t-SNARE domain containing 1
UCHL5	ubiquitin C-terminal hydrolase L5
WT1	Wilms tumour 1
WT1-AS	WT1 antisense RNA
ZBTB20	zinc finger and BTB domain containing 20
ZNF638	zinc finger protein 638

PUBLICATIONS, PRESENTATIONS AND AWARDS

Parts of this thesis have been included in the following publications and presentations:

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Presentations

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1 INTRODUCTION

1.1 Context

The world's population is rapidly ageing, with virtually all countries experiencing high growth rates in both the number and the proportion of older adults in their population due to lower birth rates and increased life expectancy. In 2015, there were 901 million people aged 60 years and over worldwide, and it is projected that this group will continue to grow to 1.4 billion by 2030 and to 2.1 billion by 2050, outnumbering children aged 0-9 years and adolescents and youth aged 10-24 years. In particular, the group of people aged 80 years and over (the 'oldest old') is experiencing the highest growth rates of all age groups, with projections suggesting this group will more than triple between 2015 and 2050 and account for one in four older adults in Europe, Northern America and Oceania by 2040 (United Nations Department of Economic and Social Affairs, 2015). A similar trend is observed in Australia; the proportion of the population aged 65 years and over increased from 12.0% to 15.3% between 1996 and 2016, and is projected to further increase to between 22.4% and 24.5% by 2061 (Australian Bureau of Statistics, 2013b, 2016). The group aged 85 years and over is expected to experience the highest growth rate and is projected to account for 4.5% to 6% of the population by 2061 (Australian Bureau of Statistics, 2013b). These demographic shifts are expected to have profound socioeconomic and health implications, and maintaining health and well-being in older age becomes a public health priority.

From a biological point of view, ageing is a complex process of gradual accumulation of molecular and cellular damage, which decreases physiological reserves and increases the risk of disease, ultimately leading to death (Kirkwood, 2008). However, the relationship between age and physical and mental functioning is not linear, and there is great inter-individual variation in experiences depending on one's genetics, environment and health behaviours. As extended life expectancy is not always accompanied by good health and quality of life, there has been much discussion of successful or healthy ageing in recent years. The MacArthur model of successful ageing proposed by Rowe and Kahn (1997) is probably the most well-known and widely adopted model in earlier ageing studies. It comprises three components: (i) low probability of disease and

disease-related disability, (ii) preservation of physical and cognitive abilities, as well as (iii) active engagement with life. Later studies have tried to expand on this concept and included various other domains in their operationalisations of successful ageing (Cosco et al., 2014; Depp & Jeste, 2006; Hung et al., 2010; Phelan & Larson, 2002). There appears to be limited consensus on how successful ageing should be defined, but the majority of the studies emphasised the maintenance of physical function or prevention of physical disability (Depp et al., 2010). A major criticism of these operationalisations of successful ageing is that the cognitive and emotional aspects of ageing are neglected. It is increasingly recognised that there is "no health without mental health" (World Health Organization, 2013); cognitive and emotional aspects of ageing are relevant not only because of the projected increase in neurodegenerative disorders, but also because cognitive and emotional health may potentially mediate health behaviours that can in turn influence physical health in later life (Depp et al., 2010).

Today the term 'healthy ageing' is more frequently used by researchers and policymakers, but likewise there is little consensus on its definition. The World Health Organisation defines it as "the process of developing and maintaining functional ability that enables well-being in older age" (World Health Organization, 2015, p. 28), which reflects a more holistic approach that encapsulates both physical and mental aspects of ageing and highlights the multidimensional nature of well-being in older age. It is evident from findings of the Global Burden of Disease project that mood disorders can greatly limit the functional ability of older adults, with depressive disorders ranked among the leading causes of disability (measured in years of healthy life lost due to disability, YLD) in populations aged 60 years and over (Table 1.1).

Table 1.1. Leading causes of disability in populations aged 60 years and over, estimated for the year 2015 (World Health Organization, 2015).

Cause	Years lost due to disability (YLD)	%
Other hearing loss	14,715,972	8.0
Back and neck pain	13,492,189	7.3
Diabetes mellitus	13,282,340	7.2
Alzheimer disease and other dementias	9,426,903	5.1
Depressive disorders	8,893,388	4.8
Uncorrected refractive errors	8,638,669	4.7
Other musculoskeletal disorders	7,464,724	4.1
Osteoarthritis	6,723,578	3.7
Other circulatory diseases	5,533,825	3.0
Chronic obstructive pulmonary disease	5,480,591	3.0

Late-life depression (LLD) is associated with various adverse health outcomes, such as higher rates of medical comorbidities, increased risk of functional and cognitive impairment, and increased suicide and non-suicide mortality (Blazer, 2003). Older adults with LLD also utilise more health care services, resulting in higher health care costs compared to their non-depressed counterpart (Katon et al., 2003; Livingston et al., 1997; Luber et al., 2001; Luppa et al., 2008), and a recent costing study conducted in Germany estimated the unadjusted means costs for a depressed older adult to be around €5,000 for a six-month period, which is more than 80% higher than that of a non-depressed older adult. This difference in health care costs remained statistically significant even after adjusting for medical comorbidities and sociodemographic factors, and was unrelated to whether these older adults received a formal diagnosis of depression from their general practitioners (Bock et al., 2016).

LLD is generally underdiagnosed and undertreated (Rodda et al., 2011). The accuracy of depression recognition by general practitioners or non-psychiatric physicians is low (Cepoiu et al., 2008; Mitchell et al., 2009), and the rate is even lower in older adults (Mitchell et al., 2010). Although LLD can be successfully treated, few older adults with LLD receive adequate treatment, with just under 30% of patients with a diagnosis given antidepressants (Luber et al., 2001), and less than 10% received counselling or psychotherapy (Unützer et al., 2003). This is further complicated by low rates of treatment-seeking behaviour (Conner et al., 2010), inadequate

dosage of antidepressant medications (Unützer et al., 1999) and treatment discontinuation due to perceived stigma (Sirey et al., 2001). However, it is estimated that even with optimal evidence-based treatment, only 34% of YLDs in depression and 37% of YLDs in dysthymia could be averted (Andrews et al., 2004).

1.2 Rationale

It is clear that LLD represents a significant public health challenge due to its association with greater disability, morbidity and mortality. The current literature suggests LLD involves a complex interplay of biological and environmental factors, such as genetic vulnerabilities, age-associated neurobiological changes, and exposure to stressful life events (Naismith et al., 2012). Despite extensive research, the exact biological underpinnings of LLD remain elusive and treatments that directly target the casual factors of LLD are unavailable. For this reason, prevention should play a key role in reducing the disease burden. Using the framework proposed in the Institute of Medicine consensus report (Mrazek & Haggerty, 1994), 'selective' and 'indicated' prevention appear to be particularly relevant for the prevention of LLD. Selective preventive strategies target subpopulations that are deemed to be at increased risk for developing LLD, for instance those who have multiple medical comorbidities or those who experience social isolation. Indicated prevention, in contrast, targets those who are already experiencing early signs or subthreshold symptoms.

Early prevention studies of LLD show directing prevention efforts toward at-risk groups, for example older adults with anxiety, chronic illnesses or functional impairment, can offer large health gains in a cost-effective way, and may halve the incidence of LLD (Schoevers et al., 2006; Smit et al., 2006; Smits et al., 2008). Without a doubt, identification of more risk factors and biomarkers associated with LLD will further facilitate preventive efforts. A closer examination of the genetic, environmental and epigenetic influences on LLD would provide more insight into the neurobiological pathways involved in the development of LLD, and may aid better identification of at-risk sub-populations at which preventive efforts should be targeted.

1.3 Objective and aims of this thesis

The primary objective of this thesis is to explore the genetic, environmental and epigenetic influences on depression in older adults. Specific aims are as follows:

1. To examine the relative contribution of genetic and environmental influences on LLD and commonly co-occurring phenotypes (anxiety, stroke and hypertension);
2. To review existing evidence for genetic variants associated with risk of depression or depressive symptoms in older adults, and to replicate associations between LLD and risk single-nucleotide polymorphisms (SNPs) identified in meta-analyses;
3. To investigate the role of early-life trauma in LLD, and explore potential gene-environment interactions; and
4. To explore the role of deoxyribonucleic acid (DNA) methylation in LLD.

1.4 Thesis outline

Following this brief introduction, Chapter 2 presents a comprehensive review of the LLD literature, including its epidemiology and clinical features, risk relationships with medical comorbidities, neurobiological correlates, as well as biological and psychosocial risk factors. The dominant theories of the pathophysiology of LLD are then introduced.

Chapter 3 provides an overview of the methodology used in this thesis. It provides descriptions of the cohorts from which subsamples were drawn for the different studies, the measures and scales used, and a brief introduction to the research designs and statistical methods employed.

The next two chapters focus on the genetic contribution to LLD. In Chapter 4, the heritability of LLD and sources of the common co-occurrence of LLD, anxiety and two vascular phenotypes are examined in a sample of older twins. Chapter 5 is a systematic review and meta-analysis of genetic association studies of LLD, which is followed by replication genetic association studies of SNPs identified in meta-analyses as major depressive disorder or LLD risk variants.

The environmental contribution to LLD is then considered. Chapter 6 examines the effects of two types of early-life trauma (childhood maltreatment and war-related trauma) as well as potential gene-environment interactions on LLD.

Chapter 7 explores the epigenetic contribution to LLD. The relationships between DNA methylation levels and LLD are investigated in a sample of older monozygotic twins.

A summary of the main findings is presented in Chapter 8, followed by a discussion of their significance and clinical implications in light of current literature. Methodological and conceptual limitations are considered. Finally, future research directions are recommended.

2 BACKGROUND

2.1 Depression in later life

Depression is a common neuropsychiatric disorder that can affect individuals of all ages worldwide. Across the lifespan, prevalence rates of depression tend to peak during adolescence, then steadily decline in young, middle and early older adulthood, before increasing again around the age of 75 (Kessler et al., 1992; Luppa et al., 2012).

2.1.1 Diagnosis

Late-life depression (LLD) is a broad term that can refer to both depressive symptoms and depressive disorders, which form a continuum ranging from the very mild to the very severe. Currently, the clinical diagnosis of depressive disorders in older adults is generally based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association, 2013) or the International Classification of Diseases (ICD-10; World Health Organization, 1992). The DSM-5 characterises major depressive disorder (MDD) as the presence of at least five out of nine symptoms, including at least one of the core symptoms of persistent depressed mood and anhedonia, over a two-week period, whereas the ICD-10 also includes reduced energy as a core symptom (see Table 2.1). Minor or subthreshold depression, on the other hand, is diagnosed when one to three additional symptoms are present alongside one of the core symptoms. It is important to note that even depressive states that do not meet the criteria for MDD, including minor depression, subsyndromal depression and dysthymia, are still clinically relevant as they are similarly associated with poorer outcomes, including increased functional disability, cognitive impairment and higher mortality rates (Han et al., 2008; Lavretsky & Kumar, 2002; Lyness et al., 2006; Penninx et al., 1999). Depressed older adults often display fewer symptoms than what is required for a DSM-5 diagnosis of MDD, and they are more likely to report cognitive and neurovegetative symptoms rather than dysphoria (Fiske et al., 2009); hence LLD is also sometimes referred to as 'depression without sadness' (Gallo & Rabins, 1999). In this thesis, the term LLD will be used to refer to all clinically significant depressive symptoms or syndromes that occur in later life (defined here as 50 years and over to be more

inclusive), and subsumes all subtypes of depression across the spectrum regardless of age of onset.

Table 2.1. DSM-5 and ICD-10 diagnostic criteria for (major) depressive disorder/episode.

DSM-5 criteria for major depressive disorder

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
    1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, hopeless) or observation made by others (e.g., appears tearful).
    2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation).
    3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.
    4. Insomnia or hypersomnia nearly every day.
    5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down).
    6. Fatigue or loss of energy nearly every day.
    7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).
    8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others).
    9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.
B. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
C. The episode is not attributable to the physiological effects of a substance or to another medical condition.
D. The occurrence of the major depressive episode is not better explained by schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder, or other specified and unspecified schizophrenia spectrum and other psychotic disorders.
E. There has never been a manic episode or a hypomanic episode.

ICD-10 criteria for depressive episode

A. At least two of the typical symptoms:
    1. Depressed mood.
    2. Loss of interest and enjoyment.
    3. Reduced energy leading to increased fatigability and diminished activity.
B. At least two of the other symptoms:

1. Reduced concentration and attention.
2. Reduced self-esteem and self-confidence.
3. Ideas of guilt and unworthiness.
4. Bleak and pessimistic views of the future.
5. Ideas or acts of self-harm or suicide.
6. Disturbed sleep.
7. Diminished appetite.

C. The symptoms last for at least 2 weeks.
D. The symptoms cause distress and difficulty in social, work or domestic activities.

Aside from standard diagnostic criteria, self-report questionnaires may be used to screen for LLD in both clinical and research settings. Screening instruments that are commonly used include the Geriatric Depression Scale (GDS) (Yesavage et al., 1982; Yesavage & Sheikh, 1986), the Centre for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977), the Beck Depression Inventory (BDI) (Beck et al., 1961), the Zung Depression Scale (Zung et al., 1965) and the Patient Health Questionnaire (PHQ) (Spitzer et al., 1994). For older adults with dementia, the Cornell Scale for Depression in Dementia (Alexopoulos et al., 1988) can be used.

2.1.2 Epidemiology

Depending on the case definition used and the age range surveyed, estimates of prevalence and incidence of depression in older adults vary greatly. While the point prevalence of late-life MDD is generally reported to be below 5%, minor depression and clinically significant depressive symptoms affect up to 16% and up to 49% of community-dwelling older adults respectively (Beekman et al., 1999; Blazer, 2003; Djernes, 2006; Polyakova et al., 2014; Volkert et al., 2013). The prevalence of depression is estimated to be higher in medical and residential aged care settings (Djernes, 2006; Polyakova et al., 2014; Seitz et al., 2010). The incidence rate of late-life MDD ranges from 0.2 to 14.1 per 100 person-years, and the incidence rate of clinically significant depressive symptoms ranges from 2.8 to 6.8 per 100 person-years (Büchtemann et al., 2012; Smit et al., 2006). These prevalence and incidence rates are likely to be an underestimation of the magnitude of the problem, as depression is commonly underdiagnosed and undertreated in older adults (Rodda et al., 2011). This is partly because the diagnosis of depressive disorders in older adults is often complicated by a range of comorbid medical conditions, physical complaints, cognitive impairment, or significant life events (e.g. bereavement or moving into a

residential aged care facility), making detection more challenging. A qualitative study that explored the perception of LLD among primary care practitioners and patients found primary care practitioners commonly viewed LLD as a product of psychosocial issues, and was thus 'understandable' and 'justifiable' in older adults. Patients appeared to share such views, and had low expectations of treatment (Burroughs et al., 2006). Moreover, the perceived stigma of a depression diagnosis may further contribute to the problem of underdiagnosis of LLD (Evans & Mottram, 2000).

2.1.3 Age of onset

Depression in late life can either be a disorder with an onset in earlier life that recurs in late life (i.e., early-onset depression, EOD) or a new disorder with recent onset (i.e., late-onset depression, LOD), and there is a body of literature suggesting that the two represent distinct clinical entities. Some studies also suggest that the late-onset group can be further divided into subgroups reflecting different aetiological pathways: (i) late-onset as a reaction to stressful life events, and (ii) late-onset with vascular risk factors (Oldehinkel et al., 2003; Van den Berg et al., 2001). A systematic review conducted in 2013 compared the symptomatology, aetiology and treatment outcomes and prognosis of EOD and LOD in 23 studies, and found that family history of mood disorders and pessimistic or suicidal thinking were both more common in EOD than in LOD (Grayson & Thomas, 2013). There was no consistent evidence for any other differences in aetiology (physical health status, vascular risk factors, life events, and personality traits), symptomatology (anxiety, psychosis or delusions, and psychomotor retardation), or treatment outcomes and prognosis (response to antidepressants, remission, recurrence, and mortality).

Grayson and Thomas' (2013) systematic review focused on clinical studies and therefore did not include studies examining the neuropsychological or neuroimaging profiles in EOD and LOD. An earlier systematic review investigated the differences in neuropsychological profiles between EOD, LOD and healthy controls in 10 studies, which showed that individuals with LOD demonstrate significant impairments in the domains of executive function, processing speed, as well as episodic and semantic memory relative to healthy controls, and they performed worse in two domains (executive function and processing speed) relative to individuals with EOD, but no

differences were observed in overall cognitive function (Herrmann et al., 2007). The pronounced impairments in executive function and processing speed in LOD suggest disruptions in frontostriatal circuits.

White matter hyperintensities (WMHs) are commonly observed in individuals with LLD. A meta-analysis comparing the presence and severity of WMHs among individuals with EOD, LOD and healthy controls showed that the odds of having periventricular and deep WMHs were 4.51 and 4.33 times greater respectively in individuals with LOD than in individuals with EOD, and age did not appear to be a confounder (Herrmann et al., 2008). The same study also showed individuals with LOD had more severe hyperintensities in the periventricular, deep and combined white matter. The white matter changes observed in the included studies were concentrated in the frontal and temporal cortex, again highlighting the role of disruptions in frontostriatal circuits.

While the use of age of onset has proved to be useful in delineating clinical subtypes of LLD that are associated with different aetiological factors, neuropsychological profiles, and neurobiological changes, there are inherent difficulties in defining what should be considered as a late age of onset. It is unclear whether onset should refer to the emergence of first symptoms of depression or of a well-defined syndrome, which is further complicated with recall bias in retrospective reporting. There appears to be no consensus on what the appropriate cut-off age should be in defining EOD versus LOD, with studies using various age cut-offs between 40 to 65 years (Sneed et al., 2006). Furthermore, the EOD versus LOD classification in a sense represents a false dichotomy, "because most studies on age of onset assume incorrectly that depressive syndromes across the life span have the same aetiology. In fact, the same person may develop depression attributable to different causes at different stages of life." (Alexopoulos, 2006, p. 1305), and individuals with a history of EOD may also develop symptoms characteristic of LOD in later life (Krishnan et al., 2004). This view is supported by the recent vascular depression consensus report, in which the authors point out that individuals with EOD may in fact be particularly at risk of developing vascular depression (a subtype of LLD that typically has an

onset at 65 years or later) with growing evidence to suggest a reciprocal relationship between vascular disease and depression (Aizenstein et al., 2016).

2.1.4 Prognosis

A meta-analysis of 12 studies conducted in community and primary care settings estimated that only around a third of participants achieved remission at the 24-month follow-up, another third remained depressed, and around 20% had died (Cole et al., 1999). Moderate and severe depression at baseline also predicted 10-year mortality after adjustment for covariates (mortality hazard ratio (*MHR*) = 1.29, 95% confidence interval (CI) 1.03-1.61 and *MHR* = 1.34, 95% CI 1.07-1.68 respectively) (Schoevers et al., 2009). The chronicity of LLD is also highlighted in more recent studies that used longer follow-up intervals of six to eight years (Beekman et al., 2002; Stek et al., 2002). Baseline depressive symptom severity, medical burden, and global psychiatric functioning were found to be consistent predictors of two-year outcome, while a history of depression also predicted outcome in the subgroup that had baseline depressive symptom severity scores in the non-depressed range, and perceived social support predicted outcome in the subgroup with baselines depressive symptom severity scores in the subsyndromal to minor depression range (Cui et al., 2008). Another study investigating whether risk factors of incident depression also predicted prognosis of depression in older adults found that a history of depression, functional limitations and incident anxiety predicted a more chronic course of depression (Schoevers et al., 2003a). One study examined recurrence rates of LLD, and the overall recurrence rate for all depressive syndromes was found to be 65.6 (95% CI 61.8-69.7) per 1000 person-years using a 2-year criterion (Luijendijk et al., 2008). While antidepressants appear to be efficacious in the treatment of late-life MDD, the treatment effect was no longer significant when a meta-analysis was stratified to include only studies using an older LLD group (i.e. employing an age cut-off of 65 or 75 years), suggesting that treatment response could possibly be reduced in individuals aged 65 years or older (Tedeschini et al., 2011).

MDD is not only closely associated with suicide in older adults (Conwell et al., 2011), other studies have shown it is also linked to a higher risk of non-suicide mortality (Schulz et al., 2002). A number of mediating mechanisms have been put forward, for example cardiovascular disease

may be triggered by psychological distress, as well as poor health behaviours such as smoking, physical inactivity, and medication non-adherence (Schulz et al., 2002).

2.1.5 Medical and psychiatric comorbidities of late-life depression

LLD often co-occurs with other medical and psychiatric conditions in late life, leading to greater levels of functional disability and higher health care costs. The following presents a review of several common medical and psychiatric comorbidities of LLD and their risk relationships.

2.1.5.1 Frailty

Frailty is a clinical syndrome in older adults characterised by increased vulnerability to impairments due to decreased reserve capacity in multiple physiological systems such that resistance to everyday or acute stressors is compromised (Brown et al., 2016; Rodríguez-Mañas et al., 2013). While there is no consensus definition of frailty to date, one operational definition is proposed by Fried et al. (2001), which conceptualises frailty as the presence of three or more of five criteria: unintentional weight loss, self-reported exhaustion, weakness, slow walking speed, and low physical activity. The literature suggests that frailty and depression represent distinct but interrelated constructs, and there is substantial overlapping of both symptoms and risk factors of the two syndromes (Mezuk et al., 2013). The prevalence of physical frailty is significantly higher in depressed older adults compared to non-depressed older adults (odds ratio (*OR*) = 2.66, 95% CI 1.36-5.24, $p = 0.004$), even after controlling for covariates (age, gender, number of comorbid chronic physical illnesses, global cognitive function, and living status). Moreover, physical frailty was positively associated with severity of depression (*OR* = 1.07, 95% CI = 1.05-1.10, $p < 0.001$) within the depressed group (Collard et al., 2014). Besides, frailty was found to partly mediate the relationship between depression and the number of somatic disease, with the association mainly driven by the exhaustion criterion in the frailty definition (Collard et al., 2015a); moreover, physical frailty predicts the onset of depressed mood, and is associated with lower rates of remission (Collard et al., 2015b).

A review of the literature suggests that this association between frailty and LLD may be bidirectional (Brown et al., 2016; Mezuk et al., 2012). Several explanations have been proposed for this frailty-depression relationship. Firstly, physiological disturbances underlying depression

as well as health behaviours associated with depression may result in an increased risk of developing frailty in depressed older adults. Secondly, frailty is commonly associated with chronic physical illnesses and functional impairment, and these may contribute to more severe depression. Lastly, there may be common underlying mechanisms that contribute to the development of both frailty and LLD (Collard et al., 2014).

2.1.5.2 Sleep disturbance

Sleep disturbance is one of the symptoms included in the diagnostic criteria for depression, and is reported in more than half and up to 90% of individuals with diagnosed depression (Tsuno et al., 2005). Alterations of sleep architecture observed in depression commonly include impaired sleep continuity and duration, decreased slow-wave sleep (sleep stages three and four), and disinhibited rapid eye movement sleep (Tsuno et al., 2005). Sleep disturbance also appears to increase with age, with the most prominent features being advanced sleep phase and reduction in amplitude of circadian rhythms of core temperature and endocrine secretion. The prevalence estimates of sleep disturbance reported in surveys conducted among community-dwelling older adults are around 30% (Livingston et al., 1993; Roberts et al., 2000). Besides playing a critical role in memory consolidation and neurogenesis, a growing body of literature suggests that sleep is also involved in mood regulation (Walker, 2009), and studies exploring the relationship between sleep disturbance and depression in older adults show that sleep disturbance is a risk factor for the onset, maintenance and recurrence of LLD (Cho et al., 2008; Livingston et al., 1993; Pigeon et al., 2008; Roberts et al., 2000).

2.1.5.3 Chronic pain

Pain is a common concern among older adults, with chronic pain, i.e. pain that persists beyond normal tissue healing time, affecting more than half of older adults, and higher rates of pain are generally observed in females than in males (McCarthy et al., 2009; Miró et al., 2007; Takai et al., 2010). Individuals with chronic pain were more likely to have a diagnosis of depression (McCarthy et al., 2009) and were more likely to experience more severe depressive symptoms (Landi et al., 2005); conversely, those with a diagnosis of depression were more likely to report the presence of pain symptoms (Bonnewyn et al., 2009). Longitudinal studies also highlight such

a reciprocal relationship between pain and depression: pain at a previous assessment predicted onset of depression, and depression at a previous assessment predicted onset of pain in community-dwelling older adults (Arola et al., 2010; Chou, 2007; Geerlings et al., 2002). This pain-depression relationship was not mediated by disability, but the relationship was found to be stronger in men than in women at the symptom level (Geerlings et al., 2002).

Comorbid pain and depression is associated with poor prognosis. Older adults with comorbid chronic pain and depression were more likely to have suicidal ideation, comorbid personality disorder, higher medical burden and less total sleep time (Meeks et al., 2008). Functional impairment as a consequence of pain also appears to mediate the association between pain severity and depressive symptoms (Mavandadi et al., 2007).

2.1.5.4 Overweight, obesity and metabolic syndrome

Overweight and obesity is usually defined using the body mass index (BMI), which is calculated by dividing the weight in kilograms by the height in metres squared. Individuals with a BMI of 25 or more are classified as overweight and those with a BMI of 30 or more are classified as obese based on international classification developed by the World Health Organization (2000). Rates of overweight and obesity in older adults are rather high, with 79.9% of men and 68.8% of women aged 65 to 74 years being overweight or obese, and 73.2% of men and 65.6% of women aged 75 years and over being overweight or obese in Australia (Australian Bureau of Statistics, 2015). Overweight and obesity represent a major risk factor for a range of medical conditions, including cardiovascular disease, type 2 diabetes mellitus, as well as certain cancers.

Cross-sectional studies have reported mixed results. BMI, waist circumference and waist-to-hip ratio were significantly associated with depressive symptoms; however, the latter two were associated only with the somatic- but not the cognitive-affective symptom cluster (Marijnissen et al., 2011). On the other hand, depressed older adults were found to have lower waist circumference compared to their non-depressed counterpart, and waist circumference was associated with both depression severity and duration-related clinical features (i.e. age of onset, duration of illness and lifetime comorbid dysthymia) (Marijnissen et al., 2014).

Longitudinal studies are more informative as they allow us to determine the direction of the association. Several studies have shown that a higher BMI or obesity at baseline predicted the presence of depression or depressive symptoms at follow-up (Roberts et al., 2003; Sachs-Ericsson et al., 2007). Moreover, a higher BMI or obesity at baseline was associated with higher risk of incident depression or depressive symptoms in men over five and 10 years in two studies (Almeida et al., 2009a; Vogelzangs et al., 2010). There have also been suggestions that the relationship may be reciprocal, but the evidence in older adults is mixed. One study reported baseline depression was associated with an increase in abdominal obesity (i.e. sagittal diameter and visceral fat) at follow-up (Vogelzangs et al., 2008), while another study reported that subgroups of older adults with more and persistent depressive symptoms tend to have lower BMI compared to those with low depressive symptoms (Kuo et al., 2011).

Metabolic syndrome is a cluster of risk factors that increases the risks of developing cardio- and cerebrovascular disease as well as diabetes. It is diagnosed when three or more of the following criteria are met, as shown in Table 2.2.

Table 2.2. Diagnostic criteria for metabolic syndrome (Alberti et al., 2009).

Measure	Categorical cut points
Elevated waist circumference	Population- and country-specific thresholds apply
Elevated triglycerides	≥ 1.7mmol/L or pharmacological treatment for elevated triglycerides
Reduced high-density lipoprotein cholesterol	< 1.0mmol/L in men and < 1.3mmol/L in women or pharmacological treatment for reduced high-density lipoprotein cholesterol
Elevated blood pressure	Systolic ≥ 130mmHg and/or diastolic ≥ 85mmHg or antihypertensive use in a patient with a history of hypertension
Elevated fasting glucose	≥ 5.5mmol/L or pharmacological treatment for elevated glucose

Studies examining the relationship between depression and metabolic syndrome have reported conflicting findings; one study found no association between MDD and metabolic syndrome, but subthreshold depression was linked to lower odds of having metabolic syndrome (Vogelzangs et al., 2009), while another study found that depressive symptoms were significantly associated with

metabolic syndrome and a range of metabolic risk factors in men (Marijnissen et al., 2013). It is likely that other factors may mediate or moderate this association; for example, Vogelzangs and colleagues (2007) found that the prevalence of metabolic syndrome was significantly higher in depressed older participants within the highest urinary cortisol tertile than in any other group, suggesting that hypothalamic-pituitary-adrenal (HPA) axis dysregulation mediates this association.

2.1.5.5 Diabetes mellitus

Diabetes mellitus is a complex metabolic condition marked by elevated levels of blood glucose. Data from the 2014-2015 Australian Health Survey indicate that the prevalence of diabetes increases rapidly with age, and the rate of diabetes in people aged 65 to 74 years and 75 to 84 years is double to three times that in middle-aged adults (Australian Bureau of Statistics, 2015). Diabetes in older adults is linked to a range of poor health outcomes, one of which is depression. A number of studies showed that diabetes is associated with both prevalent and incident depression, even after controlling for a range of potential confounders such as age and sex (Amato et al., 1996; Chou & Chi, 2005; de Jonge et al., 2006; Finkelstein et al., 2003; Mezuk et al., 2008). One population-based study, however, found that rates of depression were increased only in older adults with type 2 diabetes and comorbid diseases, but not in those with type 2 diabetes alone (Pouwer et al., 2003). Results from a meta-analysis support this diabetes-LLD link, with older adults diagnosed with diabetes significantly more likely to have LLD than those without diabetes (*OR* = 1.51, 95% CI 1.30-1.76) (Valkanova & Ebmeier, 2013). Some studies suggest this diabetes-LLD link may be bidirectional; however, hitherto it is unclear whether this association is mediated by other diabetes-related complications like vascular disease or visual impairment (Chou & Chi, 2005; Pan et al., 2010). Nonetheless, comorbid diabetes and depression is generally associated with a greater health burden, including increased risk of comorbid vascular disease, increased diabetic complications as well as functional disability (Black, 1999).

2.1.5.6 Vascular disease

Vascular disease is a collective term for various diseases of blood vessels, including coronary heart disease and stroke. There is growing evidence to suggest that the relationship between

depression and vascular disease is likely to be bidirectional. Specifically in older adults, depressive symptoms or disorders are associated with an increased risk of developing vascular diseases in otherwise healthy individuals (*OR* = 1.36, 95% CI 1.18-1.57) (Van der Kooy et al., 2007). A longitudinal study of geriatric rehabilitation inpatients also showed that baseline vascular burden predicted subsequent depression at 6- and 18-month follow-ups (Mast et al., 2004).

Coronary heart disease is the most common form of heart disease, and is caused by a gradual build-up of plaques in the coronary arteries. Baune et al. (2006) estimated the prevalence for any depression (unipolar MDD or dysthymia) in adults with coronary heart disease is 29.8%, which is significantly higher than that in adults without coronary heart disease (*OR* = 1.92, 95% CI 1.37-2.70). A meta-analysis of cohort studies on depression and coronary heart disease also concluded that depression predicts incident coronary heart disease (relative risk (*RR*) = 1.64, 95% CI 1.29-2.08) (Rugulies, 2002). Acute myocardial infarction is one of the major clinical forms of coronary heart disease, and it is estimated that almost one in five patients with acute myocardial infarction experience MDD and up to around a third experience significant depressive symptoms (Thombs et al., 2006). Post-myocardial infarction depression in older adults also appears to be an independent risk factor for early mortality after discharge (Romanelli et al., 2002), and dysthymia predicted poor cardiac outcome at 2.5-year follow-up (Rafanelli et al., 2010).

Depression affects around a third of adult stroke survivors (Hackett & Pickles, 2014), which is significantly higher than the proportion in adults without a history of stroke (*OR* = 2.50, 95% 1.46-4.28) (Baune et al., 2006). It is also associated with an increased risk of future stroke (*RR* = 1.34, 95% CI 1.17-1.54) (Dong et al., 2012). This stroke-depression association remains significant when meta-analyses only included samples with mean age of 50 and over (*OR* = 2.11, 95% CI 1.61-2.77) (Valkanova & Ebmeier, 2013), or samples with mean age of 65 years and over (hazard ratio (*HR*) = 1.30, 95% CI 1.18-1.44) (Pan et al., 2011). A history of transient ischaemic attacks was also associated with higher rates of depressive symptoms compared to those with

hypertension but no transient ischaemic attacks, or those with neither hypertension nor transient ischaemic attacks at 10-year follow-up (Hickie et al., 2003).

Other studies have tried to investigate the relationship between LLD and other vascular risk factors such as smoking, hypertension and dyslipidaemia, but the findings are less consistent. A meta-analysis showed that the studies that examined these associations had moderate to significant heterogeneity, and their pooled effects were all non-significant (Valkanova & Ebmeier, 2013). There is also some evidence to suggest that vascular risk factors are more commonly observed in older adults with the late-onset subtype of depression (Hickie et al., 2001).

2.1.5.7 Neurocognitive disorders

Depression is frequently associated with cognitive impairment, with around 20-46% of individuals with mild cognitive impairment (MCI) and around 30% of those with dementia experiencing depression (Gabryelewicz et al., 2004; Leyhe et al., 2017; Lyketsos et al., 2002). Studies of LLD consistently report significant neuropsychological deficits, most commonly observed in the domains of executive function, attention/processing speed, memory, and occasionally visuospatial abilities, and the deficits are often more pronounced in LOD (Herrmann et al., 2007; Naismith et al., 2003). Cognitive abilities are not independent of each other, and some studies have shown that such neuropsychological deficits are almost entirely mediated by impairments in processing speed (Butters et al., 2004; Nebes et al., 2000; Sheline et al., 2006). LLD cognitive subtypes may also be differentially associated with functional disability; one study examining patterns of neuropsychological impairments in LLD identified three different clusters – one with memory impairment only, another with executive dysfunction and memory impairment, and the last with attentional deficits and memory impairment – and it showed that the executive/memory impairment group was associated with a later depression age of onset as well as greater functional disability than the memory impairment only group (Lockwood et al., 2000).

While previously considered to be transient and often labelled as 'pseudodementia' or 'reversible dementia', current evidence indicates such deficits may persist even after treatment and remission of mood symptoms (Bhalla et al., 2006; Devanand et al., 2003; Nebes et al., 2003). In

fact, 18-57% of older adults with depression may have cognitive deficits severe enough to meet criteria for a diagnosis of dementia, and depressed older adults with what is considered 'reversible dementia' have an almost five times higher chance of developing 'irreversible dementia' than depressed older adults without dementia (Alexopoulos et al., 1993). The prevalence of comorbid cognitive impairment and depressive symptoms is also said to double every five years after the age of 70 years (Arve et al., 1999).

The relationship between depression and cognitive decline is complex, and a certain degree of controversy remains on whether depression represents a risk factor or a prodrome for cognitive decline. Studies examining the temporal relationship between depression and dementia have reported conflicting results. Meta-analyses reported that while most studies showed that previous diagnosis of depression predicts subsequent dementia and the risk appears to be positively associated with cumulative depression burden (i.e., greater frequency and severity of depressive episodes), around a quarter of published studies did not show any statistically significant difference in dementia risk between those with and without a previous diagnosis of depression (da Silva et al., 2013; Diniz et al., 2013a; Jorm, 2001; Ownby et al., 2006). A number of studies also explored whether EOD and LOD were associated with differential dementia risk and they similarly reported mixed findings (da Silva et al., 2013).

As far as dementia subtypes are concerned, individuals with LLD have a significantly higher risk of developing vascular dementia (VaD) than Alzheimer's disease (AD) (Diniz et al., 2013a; Lenoir et al., 2011; Newman, 1999), and the prevalence of comorbid depressive disorders is indeed higher in VaD than in AD (Castilla-Puentes & Habeych, 2010; Park et al., 2007). Individuals with VaD also have more severe depression and display more neurovegetative symptoms than those with AD when the groups were matched on dementia severity (Park et al., 2007; Sultzer et al., 1993). A longitudinal study of depressive symptoms in AD and VaD showed that the prevalence of depressive symptoms decreased in those with AD while it increased in those with VaD over time, suggesting depressive symptoms are likely more persistent and refractory to treatment in VaD than in AD (Li et al., 2001). Individuals with dementia with Lewy

bodies are also more likely to experience depression relative to those with AD (Ballard et al., 1999).

Besides dementia, baseline depressive symptoms have also been found to be associated with an increased risk of MCI as well as incident MCI (Barnes et al., 2006; Steenland et al., 2012). Individuals with higher baseline depressive symptom severity were also more likely to have multi-domain MCI than single-domain amnestic MCI, and were more likely to convert to dementia (Gabryelewicz et al., 2007). However, another prospective population-based study reported that depressive symptoms and cognitive impairment are only cross-sectionally associated (Ganguli et al., 2006). In addition, comorbid depressive symptoms in MCI also increase the rate of conversion to dementia (Barnes et al., 2006), with a large meta-analysis reporting a pooled RR of 1.28 (95% CI 1.09-1.52) in older adults with MCI who have comorbid depressive symptoms compared to older adults with MCI but no depressive symptoms (Mourao et al., 2016).

Fewer studies examined whether cognitive impairment represents a risk factor of LLD, and their pooled effect has been investigated in one meta-analysis. When only studies involving non-dementia cognitive impairment were included, the pooled effect size was non-significant, indicating there was no association between non-dementia cognitive impairment and depression. However, the prevalence of depression was significantly higher in older adults with dementia compared to those without dementia (OR = 1.82, 95% CI 1.15-2.89) (Huang et al., 2011).

2.1.5.8 Parkinson's disease

Depression occurs in around 35 to 50% of individuals with Parkinson's disease (Reijnders et al., 2008), and is the most common non-motor symptom of the disease. However, as there is considerable overlap in the core symptoms of both disorders, it is often difficult to differentiate between the two, resulting in potential over- and underdiagnosis. There is evidence to suggest that the probability of developing a depression was significantly higher in individuals with Parkinson's disease than in those with other chronic medical conditions, namely diabetes and osteoarthritis (Nilsson et al., 2002).

The hypothesis that depression is a prodromal condition of Parkinson's disease has also been explored in several studies. A systematic review of the literature showed that premorbid depression was significantly associated with Parkinson's disease in eight out of nine included studies, although all these studies were retrospective in nature (Ishihara & Brayne, 2006). Studies have reported that depressive symptoms may predate the onset of motor symptoms by as long as 36 years (Leentjens et al., 2003), with a significantly higher incidence especially within five years (Burn, 2002).

2.1.5.9 Other psychiatric disorders

Depressed older adults are more likely to have other comorbid psychiatric disorders than non-depressed controls (Laborde-Lahoz et al., 2015). Alcohol use disorders are common in older adults, and are associated with notable physical, psychological and social problems. Prevalence rates of alcohol use disorders in older adults are estimated to be up to 16% in community-based studies and up to 58% in health care settings (Blow et al., 2007; Lin et al., 2011), and alcohol use disorders are three to fourfold more common in depressed older adults than non-depressed older adults (Grant & Harford, 1995; Saunders et al., 1991). Depressed individuals with comorbid alcohol use disorders tend to have a more complicated clinical course and poorer prognosis compared to those with either disorder alone (Cook et al., 1991; Hanna & Grant, 1997). Drug use disorders, on the other hand, are rare in older adults (Blazer & Wu, 2009).

Research on the comorbidity of depressive and anxiety disorders in late life is scarce, but epidemiologic studies suggest the two are highly concomitant (Beekman et al., 2000; Braam et al., 2014; Lenze et al., 2000; Mulsant et al., 1996; Schoevers et al., 2003b). Prevalence of the comorbid anxiety-depression syndrome also appears to decrease with age (Byers et al., 2010). Comorbid late-life depressive and anxiety disorders are associated with more severe somatic symptoms, poorer social function, greater suicidality (Lenze et al., 2000), greater memory decline (DeLuca et al., 2005) and greater disability (Braam et al., 2014), although comorbid anxious-depression was not associated with a poorer antidepressant response (Nelson et al., 2009).

Similarly, only a few studies have examined the association between personality disorders and LLD, and the relationship is not well established. Early studies with small sample sizes found that older individuals with a history of MDD, particularly the early-onset subgroup, were more likely to have greater lifetime personality dysfunction (Abrams et al., 1987; Abrams et al., 1994). Moreover, a comorbidity personality disorder appeared to predict the maintenance or recurrence of depressive symptoms in depressed older adults (Morse & Lynch, 2004).

2.2 Neurobiological correlates of late-life depression

LLD is a heterogeneous neuropsychiatric disorder that likely has a multifactorial aetiology; there is increasing evidence to show that there are structural, functional and molecular alterations in the brain as well as neuropathological substrates underlying LLD (Naismith et al., 2012). A review of existing evidence on the neurobiological correlates of LLD is presented below.

2.2.1 Structural neuroimaging

2.2.1.1 Structural magnetic resonance imaging

Neurobiological abnormalities, particularly in the fronto-subcortical and limbic regions, have been hypothesised to play a role in the development of LLD, and magnetic resonance imaging (MRI) has proven to be a useful tool for the *in vivo* examination of such abnormalities in LLD.

Structural MRI can be used to study grey matter volumetric changes in specific pre-defined cortical and subcortical regions using the region of interest analysis, or globally using voxel-based morphometry. Most studies that measured whole brain volumes did not detect significant global volumetric reductions in depressed older adults (Andreescu et al., 2008; Ballmaier et al., 2008; Lloyd et al., 2004; Pantel et al., 1997; Sheline et al., 1996), and a meta-analysis of 14 studies showed that the pooled effect was not significant (Sexton et al., 2013). Atrophy of the frontal lobe as well as of subregions of the prefrontal cortex, including the anterior cingulate cortex and the orbitofrontal cortex, has been observed in numerous studies (Andreescu et al., 2008; Kumar et al., 2000; Lavretsky et al., 2007; Lehmbeck et al., 2008; Taylor et al., 2007a). These frontal volumetric reductions may be more strongly associated with the late-onset subtype, as one study reported significantly smaller frontal lobe volumes in LOD relative to EOD and controls

(Almeida et al., 2003), whereas two other studies comparing EOD to controls found no significant differences in frontal lobe volumes (Janssen et al., 2004; Weber et al., 2010).

The hippocampus is the most frequently studied region of interest in relation to LLD due to its role in mood regulation as well as its connections to other brain regions implicated in depression including the thalamus, amygdala, basal ganglia, and prefrontal cortex. Hippocampal volumetric reductions are common in studies of LLD (Andreescu et al., 2008; Ballmaier et al., 2008; Lim et al., 2012; Sheline et al., 1996), although findings are inconsistent in regard to whether hippocampal volumes differ between EOD and LOD (Ballmaier et al., 2008; Janssen et al., 2007; Lloyd et al., 2004). A recent meta-analysis showed that both EOD and LOD are associated with significant hippocampal volumetric reductions, but the volumetric changes are greater in LOD than in EOD (Geerlings & Gerritsen, 2017). There may also be an age of onset by genotype interaction, with smaller hippocampal volumes observed in solute carrier family 6 member 4 (*SLC6A4*) 5-HTTLPR L/L homozygotes with LOD but in S/S homozygotes with EOD in one study (Taylor et al., 2005). Furthermore, specific depressive symptom dimensions appear to be differentially associated with grey matter volumes, as a vertex-wise analysis study showed that scores on the depressed mood subscale of the CES-D were positively associated with grey matter volumes in the left inferior temporal lobe after correcting for multiple comparisons (McLaren et al., 2017).

In addition to global hippocampal volumetric changes, studies have also examined hippocampal shape differences in LLD using computational mapping techniques. Comparing depressed older adults and controls, Zhao and colleagues (2008) found shape differences in the left hippocampus that roughly correspond to contraction in the dentate gyrus and the CA4 subfield, and expansion in the subiculum and the CA2 and CA3 subfields. When the depressed group was further divided into remitted and non-remitted subgroups, it revealed the significant shape differences distinguished the depressed non-remitted group from the remitted and controls groups. Another study showed significant bilateral regional volumetric reductions that correspond to contractions in the anterior CA1 to CA3 subfields and the subiculum in LLD compared to controls, but comparisons between EOD and LOD also showed more pronounced reductions in the anterior

aspects of the subiculum and the lateral-posterior aspects of the CA1 subfield in LOD (Ballmaier et al., 2008).

A few studies have examined the association between cortical thickness and LLD, but they reported contradictory results. While two earlier studies found no effect of LLD on cortical thickness , two other studies observed cortical thinning in the frontal, parietal, temporal and cingulate regions (Lim et al., 2012; Mackin et al., 2013).

On the other hand, the presence of WMHs or white matter lesions is one of the most consistently reported finding in the LLD neuroimaging literature. WMHs, also referred to as leukoaraiosis or leukoencephalopathy in the early LLD literature, are increased signal intensities on T2-weighted fluid attenuated inversion recovery magnetic resonance imaging. These cerebral white matter lesions generally predate the onset of depressive symptoms but are in fact also present in the majority of community-dwelling older adults, with age being the biggest predictor of total WMH burden (de Leeuw et al., 2001; Liao et al., 1997; O'Brien et al., 1996a; Söderlund et al., 2003; Ylikoski et al., 1995). WMHs also appear to be associated with the persisting cognitive deficits in LLD, specifically in the domains of memory and executive function (Köhler et al., 2010).

WMHs can be further classified into periventricular hyperintensities and deep WMHs based on their location in the brain. Periventricular hyperintensities are located adjacent to the lateral ventricles whereas deep WMHs are located in the subcortical regions. The severity of these WMHs was traditionally rated visually using standardised scales such as the Fazekas scales (Fazekas et al., 1987), the Scheltens scale (Scheltens et al., 1993) or with reference to the dilation of Virchow-Robin spaces, but volumetric measurements are now typically utilised in most WMH studies. Deep WMHs have consistently been found to be more common and/or more severe in LLD (O'Brien et al., 1996b; Thomas et al., 2004), but some studies also suggest there are increased and/or more severe periventricular hyperintensities in LLD (Coffey et al., 1990; de Groot et al., 2000; Herrmann et al., 2008). Deep WMHs (both large and punctate) in depressed older adults are more likely to be ischaemic in nature, and are primarily located in the dorsolateral prefrontal cortex, while punctate deep WMHs in the control group are usually non-ischaemic (Thomas et al., 2002a). The causes for periventricular hyperintensities, however,

appeared to be similar for both depressed and non-depressed older adults, namely (i) ependymal loss, (ii) sudden contrast in myelination between adjacent fibre tracts and fibre loss in the WM, and (iii) ischaemic demyelination and tissue loss (Thomas et al., 2002b; Thomas et al., 2003). A meta-analysis that compared the presence and severity of WMHs in EOD and LOD showed that those with LOD are approximately four times as likely to have periventricular hyperintensities and deep WMHs as their early-onset counterpart; however, these subgroups did not differ significantly in terms of WMH severity (Herrmann et al., 2008).

It is possible that the strategic location of WMHs is of greater importance than total volume, and WMHs disrupting frontal-subcortical projections or the dorsolateral prefrontal circuit have been implicated in vascular depression. When the WMH burden in individuals with LLD was compared to controls, increased WMHs were detected in the white matter tracts within the dorsolateral prefrontal circuit (Sheline et al., 2008) and in the medial orbital prefrontal cortex (MacFall et al., 2001) in the LLD group.

2.2.1.2 Diffusion tensor imaging

Diffusion tensor imaging (DTI) is a MRI technique that can be used to assess white matter microstructure through measuring the diffusion of water molecules in white matter fibre tracts, and allows for the structural integrity of pathways involved in various neural networks to be examined. There are four main indices that can be derived from DTI – fractional anisotropy (FA) measures the degree of directionality, mean diffusivity measures the average water diffusion regardless of directionality, radial diffusivity measures myelin integrity, and axial diffusivity measures axon integrity. These can be assessed either locally in subjectively defined regions using region of interest analysis or globally with voxel-based analysis or tract-based spatial statistics.

DTI studies of LLD consistently report widespread reductions in FA in depressed older adults. Most studies found reduced FA predominantly in frontal and temporal regions (Alexopoulos et al., 2009; Bae et al., 2006; Nobuhara et al., 2006; Yang et al., 2007; Yuan et al., 2007), while a few studies reported reductions in FA in the corpus callosum, the cingulate cortex and/or the cingulum bundles (Alves et al., 2012; Bae et al., 2006; Mettenburg et al., 2012) and another two reported diffuse reductions in FA across multiple brain regions (Reppermund et al., 2014; Sexton

et al., 2012). Two other studies, however, reported no significant differences in FA between the depressed and non-depressed groups (Bezerra et al., 2012; Colloby et al., 2011). One study that excluded regions with WMHs found that even normal-appearing white matter can have microstructural abnormalities when investigated using DTI (Shimony et al., 2009). A recent meta-analysis showed that when results were pooled together, significant FA reductions were found in the frontal lobe, the corpus callosum, the uncinate fasciculus and the cingulum (Wen et al., 2014). Studies that also used other DTI indices provided some evidence for increased mean, radial and axial diffusivity in the corpus callosum, uncinate fasciculus or the cingulum (Charlton et al., 2014; Mettenburg et al., 2012). Subthreshold depressive symptoms also appear to be associated with reduced FA and increased radial and axial diffusivity (Allan et al., 2016).

2.2.2 Functional neuroimaging

2.2.2.1 Resting-state functional magnetic resonance imaging

Disruptions to the frontostriatal and limbic networks have been hypothesised to underlie LLD. Resting-state functional MRI can be used to investigate the degree of functional connectivity in various brain networks. It is considered that LLD involves alterations across multiple resting state networks, such as the default mode network, the affective network, and the cognitive control network. Most studies suggest that LLD is associated with increased functional connectivity in the default mode network (Alexopoulos et al., 2012; Andreescu et al., 2013; Wu et al., 2011) and decreased functional connectivity in the cognitive control network (Aizenstein et al., 2009; Alexopoulos et al., 2012) and the affective network (Wu et al., 2011). However, two other studies observed widespread or global increased connectivity (Bohr et al., 2013; Kenny et al., 2010) and another found no significant differences in functional connectivity in any of the networks (Sexton et al., 2012).

Resting-state functional connectivity also appears to be correlated with white matter macro- and microstructure, with significant negative correlations observed between resting-state functional connectivity in the medial frontal region and normalised WMH volume in both depressed and non-depressed older adults (Wu et al., 2011), as well as between resting-state functional

connectivity in the left vendromedial prefrontal cortex-caudate and fractional anisotropy in the left uncinate fasciculus in older adults with remitted MDD (Steffens et al., 2011).

2.2.2.2 Positron emission tomography

Positron emission tomography (PET) is an *in vivo* imaging technique that can be used to quantify perfusion or metabolism through the detection of γ radioactivity that is emitted by a radioligand, and it offers high temporal and spatial resolution. The use of different radioligands allows for various biological processes to be visualised, including activation of microglia, beta-amyloid deposition, as well neurotransmitter receptor binding (Hirao & Smith, 2014).

Only two studies have examined the alterations to cerebral glucose metabolism in LLD relative to controls using [^{18}F]fluorodeoxyglucose PET, and the findings were contradictory. Kumar et al. (Kumar et al., 1993) found hypometabolism in most neocortical, subcortical and paralimbic regions assessed, while Smith et al. (Smith et al., 2009b) reported hypermetabolism in both anterior and posterior cortical regions, with cerebral cortical metabolism positively correlated with severity of depressive symptoms. Treatment-resistant late-onset MDD was found to be associated with decreased metabolism in the prefrontal, cingulate, temporal and parietal regions, as well as increased metabolism in other regions including the dorsal frontal region, occipital pole, cerebellum and basal ganglia (Fujimoto et al., 2008). Other studies generally observed decreased metabolism in the anterior cortical and limbic regions and increased metabolism in the posterior cortical regions and cerebellum following treatment (Diaconescu et al., 2011; Smith et al., 2002; Smith et al., 1999), although this was not the case in another study (Smith et al., 2009c). Lower baseline and greater reductions in metabolism appear to be associated with better antidepressant response (Smith et al., 2011; Smith et al., 2009a).

Besides cerebral glucose metabolism, cerebral blood flow can be measured using the [^{15}O]water ligand. Cross-sectionally, mean depressive symptom scores were found to be associated with regional cerebral blood flow (rCBF) mainly in frontotemporal regions and the cerebellum in a sample of dementia-free older adults with subthreshold depression. Longitudinally, rCBF decreases were observed in frontal regions in those with higher depressive symptom scores, and in temporal regions in men only (Dotson et al., 2009). One small study also assessed rCBF using

an activation paradigm (using a word generation task), and observed activation deficits in the dorsal anterior cingulate gyrus and hippocampus bilaterally in depressed older adults compared to controls, and their memory scores were correlated with both resting and activation rCBF in the hippocampus (de Asis et al., 2001).

PET has also been used to examine monoaminergic function in LLD. Investigations of serotonergic function in LLD has been carried out using the [^{11}C]WAY 100635 and [^{18}F]altanserin ligands. Reduced serotonin (5-HT)$_{1A}$ receptor binding in the dorsal raphe nucleus was observed in a group of depressed older adults relative to controls, with binding potential correlated with severity of depressive symptoms (Meltzer et al., 2004). The findings with regards to 5-HT$_{2A}$ receptor binding, however, are inconsistent, with Meltzer et al. (1999) reporting no alterations of 5-HT$_{2A}$ receptor binding in LLD and Sheline et al. (2004) reporting reduced 5-HT$_{2A}$ receptor binding in LLD. Antidepressant medications may mediate the association between 5-HT$_{2A}$ binding potential and LLD, as the untreated group showed greater reductions in 5-HT$_{2A}$ receptor binding. There were no significant differences in 5-HT$_{2A}$ receptor binding between EOD and LOD (Sheline et al., 2004). While alterations in other neurotransmitter systems such as the dopaminergic, glutamatergic, GABAergic systems have also been implicated in depression, there are no published studies to date that examined their function in LLD.

On the other hand, [^{11}C]PK11195 is a radioligand that selectively binds to the peripheral benzodiazepine receptor (also known as the translocator protein) and is a marker for neuroinflammation, specifically microglial activation. Increased binding of [^{11}C]PK11195 has previously been reported in individuals with stroke and AD (Edison et al., 2008; Gerhard et al., 2005; Pappata et al., 2000). A small study recently showed there was greater [^{11}C]PK11195 PET binding in older adults with DSM-IV MDD in the left subgenual anterior cingulate cortex and right parahippocampus relative to controls (Su et al., 2016).

Furthermore, PET imaging has also been used to examine various types of neuropathology *in vivo*. AD pathology has been examined in LLD using the Pittsburgh compound B. Butters et al. (2008) showed that there were varying degrees of Pittsburgh compound B retention in remitted late-life MDD, with elevated Pittsburgh compound B retention observed in those with

concurrent MCI. Untreated LLD without concurrent MCI, on the other hand, was associated with greater amyloid beta deposition in the anterior cingulate gyrus, superior and middle front and temporal gyri, and precuneus; greater amyloid beta deposition also correlated with poorer neuropsychological performance as well as less cognitive improvement following treatment (Marano et al., 2013). More recently, the [^{18}F]FDDNP ligand has been used in studies to examine protein binding of amyloid beta and tau in LLD. Depression scores were correlated with FDDNP binding in the lateral temporal region in middle-aged and older adults with concurrent MCI, and in the medial temporal region in those without concurrent MCI (Lavretsky et al., 2009), whereas late-life MMD was associated with greater FDDNP binding in the posterior cingulate and lateral temporal regions (Kumar et al., 2011).

In summary, there are limited PET studies of LLD to date and the findings are far from conclusive. The ongoing development of radioligands for other neurotransmitter systems, such as [^{11}C]MRB that binds to the norepinephrine transporter (Ding et al., 2010) may further elucidate the pathophysiological mechanisms involved in LLD.

2.2.2.3 Single-photon emission computed tomography

Single-photon emission computed tomography can be used to quantify resting-state changes in rCBF. rCBF can be directly assessed using the ^{133}Xe radioligand or indirectly using the ^{99m}Tc-hexamethylpropylene amine oxime radioligand. While studies seem to disagree on whether there is global cerebral hypoperfusion in LLD (Lesser et al., 1994; Nobler et al., 2000; Upadhyaya et al., 1990), significant reductions in relative rCBF bilaterally in the prefrontal, temporal and parietal cortices, the hippocampus and the caudate nucleus were demonstrated in late-life MDD when compared to healthy controls (Awata et al., 1998; Ishizaki et al., 2008; Nobler et al., 2000).

When the depressed group was stratified by age of onset, Ebmeier et al. (1998) found decreased cerebral perfusion in the temporal lobe of those with LOD relative to both those with EOD as well as controls. Some studies observed interaction effects with sex, for instance greater reductions in cerebral perfusion were observed in the whole brain (Lesser et al., 1994) as well as specifically in the frontal and temporal cortices, anterior cingulate, caudate and thalamus (Curran et al., 1993) in older depressed men. The degree of cerebral perfusion, however, was not

always associated with clinical features such as age of onset or severity of depressive symptoms (Awata et al., 1998; Curran et al., 1993; Navarro et al., 2001).

2.2.3 Post-mortem studies

Influenced by the monoamine hypothesis of depression, two early post-mortem studies investigated neurotransmitter receptor binding sites in depressed older adults. No alterations in 5-HT_{1A} binding sites were found, but reductions in the number of 5-HT_2 binding sites were observed in frontal, temporal and parietal tissues (Bowen et al., 1989). Crow and colleagues (1984), on the other hand, found no significant differences in 5-HT binding sites between depressed older adults and controls.

Relatively few studies have investigated the neuropathological correlates of LLD, and the results are inconsistent most likely due to the great heterogeneity in methodology as well as populations sampled. Their findings are summarised in Table 2.3 below (in the case of overlapping samples, only the study with the largest sample is included). In brief, LLD was not associated with most of the pathologies examined, including amyloid pathology, α-synucleinopathy, tauopathy or vascular pathology. Among the significant findings, neuronal loss in subcortical structures (substantia nigra, nucleus basalis, raphe nucleus and locus coeruleus) (Tsopelas et al., 2011), greater AD pathology burden (Sweet et al., 2004; Wilson et al., 2016), more Lewy bodies in the substantia nigra and locus coeruleus (Tsopelas et al., 2011), and a greater degree of atheroma (Thomas et al., 2001) have been observed in the LLD group relative to controls. Both Sweet et al. (2004) and Wilson et al. (2016) included participants with dementia, which may have been a confounding factor. Few studies utilised more sensitive vascular and inflammatory markers in LLD. There is also one report of increased expression of intracellular adhesion molecule 1 (*ICAM-1*) and vascular cell adhesion molecule 1 (*VCAM-1*) in the dorsolateral prefrontal cortex, suggesting there is increased ischaemia-induced inflammation in LLD (Thomas et al., 2002a). It is important to bear in mind that while these findings provide some support for the vascular depression hypothesis, there has not been any replications of these studies to date.

Despite the crucial role of glial cells in neurogenesis and synaptic plasticity, only a small number of post-mortem studies examined changes in glial density in LLD. Among these limited studies,

only one study (overlapping samples in two papers) reported a reduction in glial density in the amygdala in LLD (Bowley et al., 2002; Hamidi et al., 2004). No differences in glial density was observed in other regions including the entorhinal cortex, the dorsolateral prefrontal cortex, the orbitofrontal cortex, the caudate nucleus or the anterior cingulate cortex (Hamidi et al., 2004; Khundakar et al., 2009; Khundakar et al., 2010, 2011a; Khundakar et al., 2011b; Rajkowska et al., 2005). Studies that tested potential differences in glial density between EOD and LOD also found no significant differences (Khundakar et al., 2009; Rajkowska et al., 2005).

Table 2.3. Summary of clinicopathological studies of LLD.

Study	Cases	Controls	Criteria / instrument used to assess depression	Brain weight / neuronal loss	AD pathology	Lewy bodies	Braak stage	Vascular pathology	Other
Hendricksen et al. (2004)	n=14, age at death=71.0±8.6 years, 71% female	n=10, age at death=77.5±6.7 years, 60% female	CES-D>10 & DSM-III-R MD or DSM-IV MDD	n.s.	n.s.				
Jellinger et al. (2009)	n=12, age at death=73.7±6.5 years, 75% female	n=27, age at death=72.8±5.7 years, 63% female	DSM-IV or ICD-10 depression or bipolar disorder	n.s.		?	n.s.		
Royall & Palmer (2013)	n=436, age at death=85.9±5.3[a]		CES-D		n.s.	n.s.		n.s.	
Santos et al. (2010)	n=38, age at death=79±6.1, 58% female	n=29, age at death=80±8.6, 48% female	DSM-IV MD with first episode at age 65 years or older					n.s.	Deep white matter or periventricular white matter demyelination n.s.; focal or diffuse gliosis n.s.
Sweet et al. (2004)	n=10, age at death=85.1±7.4, 90% female	n=56, age at death=81.2±6.8, 50% female	DSM-IV MDD or BD		+	n.s.		n.s.	Other degeneration n.s.

Study	Cases	Controls	Criteria / instrument used to assess depression	Brain weight / neuronal loss	AD pathology	Lewy bodies	Braak stage	Vascular pathology	Other
Syed et al. (2005)	n=9, age at death>70, 57% female	n=18, age at death>70, 57% female	GMS-AGECAT>3	n.s.	n.s.			n.s.	
Thomas et al. (2001)	n=20, age at death=75.0±7.4, 65% female	n=20, age at death=74.2±7.5, 65% female	DSM-IV MD		n.s.	Nil	n.s.	Atheroma +, micro-vascular n.s.	
Tsopelas et al. (2011)	n=36, age at death=82.3±7.5, 58% female	n=117, age at death=85.0±7.5, 51% female	GMS-AGECAT>3	Brain weight n.s.; neuronal loss +	n.s.	+	n.s.	n.s.	
Wilson et al. (2016)	n=657, age at death=87.9±6.7[a]		DSM-III MD with subset of questions from DIS; 10-item CES-D		Plaques + for MD but not depressive symptoms; NFTs n.s.	n.s.		n.s.	

[a] Separate demographic information for cases versus controls have not been reported in these studies.

Abbreviations: AGECAT – Automated Geriatric Examination for Computer Assisted Taxonomy; AD – Alzheimer's disease; BD – bipolar disorder; CES-D – Centre for Epidemiologic Studies Depression Scale; DIS – Diagnostic Interview Schedule; DSM – Diagnostic and Statistical

Manual of Mental Disorders; GMS – Geriatric Mental State Examination; LC – locus coeruleus; MD – major depression; MDD – major depressive disorder; NFT – neurofibrillary tangles; n.s. – non-significant

2.3 Risk factors for late-life depression

Despite major advances in neuroimaging techniques in the past two decades that have provided important insights into the neurobiological disruptions associated with LLD, it is unclear whether such neurobiological alterations represent the cause or effect of the disorder, and understanding of the aetiological mechanisms underlying LLD is still limited. There is now increasing recognition that multiple biopsychosocial risk factors contribute to the development of LLD (including but not limited to genetics, chronic disease, and stressful life events), with complex interactions between these various risk factors that determine an individual's vulnerability and resilience to the disorder. Some of the risk factors that have been examined more often in the literature are reviewed below.

2.3.1 Genetic risk factors

Family, twin and adoption studies consistently demonstrate that there is a significant genetic component in depression, with the heritability of MDD in adulthood estimated at 37% (Sullivan et al., 2000). Five published studies examined the heritability of LLD and reported modest to moderate heritability (Carmelli et al., 2000; Gatz et al., 1992; Jansson et al., 2004; Johnson et al., 2002; McGue & Christensen, 1997), suggesting that genetics explain a significant proportion of the variance in LLD.

Genetic polymorphisms associated with depression in early and mid-life have been studied extensively, yet few candidate gene studies have been conducted for LLD. Most of these studies have focused on the most obvious candidates, for example *SLC6A4* 5-HTTLPR, brain derived neurotrophic factor (*BDNF*) Val66Met, methylenetetrahydrofolate reductase (*MTHFR*) C677T as well as apolipoprotein E (*APOE*) ε2/ε3/ε4, for their associations with MDD in non-geriatric samples as well as with conditions commonly comorbid with LLD such as vascular disease and dementia.

The *SLC6A4* 5-HTTLPR polymorphism involves a 44-base pair insertion (L allele) of deletion (S allele) in the promoter region of the gene, and the S allele is associated with lower transcription of the 5-HT transporter, resulting in reduced 5-HT uptake. Most studies that directly examined

the relationship between 5-HTTLPR genotype and depression found no significant association (Mendes et al., 2013; Taylor et al., 2007a; Zannas et al., 2013). In contrast, one study observed an association between the S/S genotype and depression (including both past and present depression) in older adults, with higher frequency of S/S carriers particularly in early-onset depression (Grünblatt et al., 2006b).

BDNF is a neurotrophin that plays a critical role in the regulation of neuronal survival and plasticity, and the *BDNF* Val66Met Met allele is associated with decreased activity-dependent secretion of BDNF (Egan et al., 2003) and an increased risk of LLD (Hwang et al., 2006a; Lin et al., 2009; Taylor et al., 2007b) although some studies failed to replicate this finding (Kanellopoulos et al., 2011; You et al., 2010). The T allele of the *MTHFR* C677T polymorphism is associated with reduced enzyme activity and elevated plasma homocysteine, especially in the context of impaired folate status (Ueland et al., 2001). A number of studies have examined the C677T polymorphism in relation to LLD, but they generally failed to find any significant difference in depression status between the genotype groups (Almeida et al., 2008; Chen et al., 2005; Pan et al., 2009).

APOE encodes a protein involved in lipid metabolism. It is well established in the literature that the ε4 allele confers an increased risk for AD. Studies examining the *APOE*-LLD association generally reported negative results (Harwood et al., 1999; Steffens et al., 2003a), but some studies found significant associations with certain subtypes of LLD. For example, a higher frequency of ε3/ε4s was observed in LOD compared to EOD (Krishnan et al.), and a higher frequency of ε4 allele carriers was found in LLD with psychotic features compared to LLD without psychotic features or healthy controls (Zubenko et al., 1996).

The lack of significant results found in these candidate gene studies can often be attributed to their small sample sizes and hence lack of statistical power. Moreover, as the aetiology of depression is believed to be multifactorial, and the genetic contribution of each associated genetic polymorphism is expected to be modest at best. Most of the reported candidate genetic association studies probably are underpowered to detect these very small effect sizes.

Genome-wide association studies (GWAS) of depression generally use non-geriatric cohorts, and the only GWAS of depressive symptoms that involved predominantly older cohorts is Hek et al. (2013). This GWAS included a total of 51,258 individuals at the meta-analysis stage, and only identified one SNP (rs40465 in the 5q21 region) that reached genome-wide significance.

2.3.2 Gene-environment interactions

More than a decade ago, a landmark study discovered that the adverse effects of stressful life events and childhood maltreatment on depression are moderated by the *SLC6A4* 5-HTTLPR genotype (Caspi et al., 2003). It showed that individuals with the S/S genotype were more sensitive to the adverse effects of stressful life events and were particularly susceptible to depression. This has led to a surge in interest in potential gene-environment (GxE) interactions contributing to susceptibility to psychiatric disorders. However, meta-analyses have shown that subsequent studies were not always able to replicate this finding (Munafò et al., 2009; Risch et al., 2009). In contrast, a later meta-analysis suggested the type of stressor may influence the strength of the GxE effect, as when the analysis was stratified by the type of stressor, there was strong evidence for an association between the S allele and increase stress sensitivity in those who have been exposed to childhood maltreatment as well as in those with medical conditions, while the evidence for stressful life events was only marginal (Karg et al., 2011). Limited GxE studies have been carried out for LLD, providing only preliminary evidence for 5-HTTLPR x stress (Goldman et al., 2010; Kim et al., 2007), *BDNF* x stress (Kim et al., 2007), and *GR* x stress interactions (Bet et al., 2009). A three-way 5-HTTLPR x *BDNF* x stress interaction has also been observed in one of the studies (Kim et al., 2007).

2.3.3 Potential epigenetics mechanisms

Early-life stressful events or trauma appear to be the most replicated risk for depression in the GxE studies. More recent research has suggested that environmental exposures can induce changes in the genome without alterations to the deoxyribonucleic acid (DNA) sequence through mechanisms like DNA methylation and histone modification, which can influence gene activity and expression, leading to life-long predisposition to depression.

Only two studies have examined DNA methylation in LLD thus far, both of which employed the candidate gene approach and investigated *BDNF* promoter methylation. Both studies found significant associations between *BDNF* promoter methylation at some CpG sites and prevalent and incident LLD. However, whether the associations are mediated by *BDNF* polymorphisms remain unclear (Januar et al., 2015a; Kang et al., 2015).

No epigenome-wide association studies (EWAS) of LLD have been conducted to date. There are several epigenome-wide DNA methylation studies of adult depression (Byrne et al., 2013; Cordova-Palomera et al., 2015; Davies et al., 2014; Sabunciyan et al., 2012; Uddin et al., 2010), but they reported diverging results, probably due to methodological differences such as the use of white blood cells versus peripheral blood, sampling methods, how the phenotype was defined, and the covariates controlled for in the analyses.

2.3.4 Biological and psychosocial risk factors

Aside from the aforementioned factors that may be associated with vulnerability to LLD, other biological factors may also contribute to an individual's vulnerability. Studies have reported older age (Schoevers et al., 2000), female sex (Cole & Dendukuri, 2003), chronic disease including stroke, cardiovascular disease, hypertension, diabetes, and sensory impairments (Beekman et al., 1995; Huang et al., 2010; Petersson et al., 2014; Schoevers et al., 2000; Thompson et al., 2001; Valkanova & Ebmeier, 2013), physical or functional disability (Beekman et al., 1995; Chang et al., 2016; Prince et al., 1998; Schoevers et al., 2000), physical pain (Chang et al., 2016), low birth weight in males (Thompson et al., 2001), as well as sleep disturbances (Chang et al., 2016; Cole & Dendukuri, 2003) all appear to be significant biological risk factors of LLD.

Alternatively, there are a number of psychosocial factors that are associated with an increased risk of LLD. For instance, subjective or objective socioeconomic status (Chang et al., 2016; Thompson et al., 2001), bereavement (Cole & Dendukuri, 2003; Thompson et al., 2001), low social support (Beekman et al., 1995; Prince et al., 1998), social isolation (Prince et al., 1998; Thompson et al., 2001) and loneliness (Beekman et al., 1995) have been identified as significant psychosocial risk factors of LLD.

The research is scarce for protective factors of LLD; however, certain risk factors for LLD are potentially modifiable through lifestyle changes, examples include physical inactivity, smoking, risk drinking, and abnormal BMI (underweight, overweight or obese) (Almeida et al., 2013; Chang et al., 2016).

2.4 Theories on the pathophysiology of late-life depression

2.4.1 The monoamine hypothesis

Since the discovery of monoamine oxidase inhibitors and tricyclics as effective antidepressants in the 1960s, the depression literature has largely been dominated by the monoamine hypothesis. It postulates that deficits in serotonergic, noradrenergic and/or dopaminergic neurotransmission may underlie depression (Hirschfeld, 2000). While research from the past few decades to a certain extent supports the theory that monoaminergic neurotransmitter systems play an important role in the pathogenesis of depression, the exact mechanisms involved remain unclear. In recent years, newer theories extending beyond the monoaminergic neurotransmitter systems have been proposed, with vascular disease, neuroendocrine dysregulation, immuno-inflammatory processes and reduced neuroplasticity and neurogenesis implicated.

2.4.2 The vascular hypothesis

As early as 1905, the German psychiatrist Gaupp proposed that a certain form of depression with first onset after the age of 45 years may be secondary to arteriosclerosis (Gaupp et al., 2000). He observed that the presentation and course of this subtype of depression are varied, and the depressive disorder may be accompanied by cognitive decline, neurological deficits as well as other psychiatric symptoms. Subsequent clinical and epidemiological studies have regularly reported high comorbidity between vascular risk factors, vascular disease and depression, and the silent stroke and leukoencephalopathy literature further highlighted cerebrovascular changes as a possible cause for a subtype of depression commonly observed among older adults, which Alexopoulos and colleagues (1997) coined 'vascular depression'. The vascular depression hypothesis postulates that cerebrovascular disease confers vulnerability to, triggers and maintains this subtype of depression in older adults, possibly through disruption of cortico-

striato-pallido-thalamo-cortical pathways or their modulating systems (Alexopoulos et al., 1999; Alexopoulos et al., 1997).

Over the past two decades, researchers have attempted to further refine the notion of vascular depression. Krishnan et al. (1997) classified 'MRI-defined vascular depression' as the presence of deep white matter hyperintensities or subcortical grey matter lesions rated as a two or above using a modified Fazekas classification system (Fazekas et al., 1987; Greenwald et al., 1996; Krishnan et al., 1993). When this vascular depression group was compared to a non-vascular depression group, individuals in the vascular depression group were more likely to be aged 60 years or over, to display no psychotic features, and to have first onset after the age of 40 years. Krishnan and colleagues later examined specifically the subtype of depression due to subcortical ischaemic changes, again defined based on lesion severity on MRI, and found that individuals with 'subcortical ischaemic depression' were older, had a later age of depression onset, and were less likely to have a positive family history of psychiatric disorders compared to those with non-subcortical ischaemic depression (Krishnan et al., 2004). In contrast, Alexopoulos et al. (2001) defined a 'depression-executive dysfunction syndrome' arising from disruptions to frontostriatal pathways in the context of vascular disease, which is characterised by reduced verbal fluency, impaired visual naming, psychomotor retardation, apathy, paranoia, as well as a very mild vegetative syndrome. Depression with executive dysfunction has been shown to be associated with poor antidepressant response, relapse, recurrence, and residual depressive symptomatology (Alexopoulos et al., 2000). Although several earlier studies failed to find evidence to support vascular depression as a distinct subtype of LLD (McDougall & Brayne, 2007; Naarding et al., 2009; Thuile et al., 2007), the latest consensus report argues there is now considerable evidence that vascular and non-vascular depression show distinct clinical manifestations (Table 2.4) (Aizenstein et al., 2016). However, further work is undoubtedly required to fully establish the notion of vascular depression as a distinct subtype of LLD, as there is currently a lack of consensus in its precise definition and its underlying mechanism are still poorly understood. It is believed that multiple systems and processes are involved, and cerebrovascular lesions represent only one piece of the complex puzzle of LLD (Aizenstein et al., 2016).

Table 2.4. Clinical features associated with vascular versus non-vascular depression (Aizenstein et al., 2016).

Vascular depression	Non-vascular depression
▪ Depression occurring at age 65 years or later ▪ Absence of family history ▪ Executive dysfunctions, loss of energy, subjective feeling of sadness, anhedonia, psychomotor retardation, motivational problems, reduced processing speed and visuospatial skills, deficits in self-initiation, lack of insight; depressive symptomatology may not meet criteria for any mood disorder requested in DSM-V	▪ Depression occurring at age 50 to 60 years ▪ Occasional family history ▪ Sadness, depression according to DSM-V diagnostic criteria, increased suicidality, reduced verbal fluency
▪ Higher cardiac illness burden, increased rates of vascular risk factors (hypertension, etc.) ▪ Higher risk for cognitive decline and progression to dementia ▪ Fluctuating course of cognitive impairment due to progression of white matter hyperintensities	▪ Lower or same cardiac illness burden and rates of vascular risk factors (hypertension, etc.) ▪ Lower or similar risk for cognitive decline and progression to dementia
▪ Greater treatment resistance and poorer outcome ▪ Associated with increased mortality	▪ Lower or same treatment resistance and outcome (?)

2.4.3 The glucocorticoid cascade hypothesis

The HPA axis is the major neuroendocrine system that mediates the effects of stress within the central nervous system. In face of stressors, the HPA axis is activated and a feed-forward neuroendocrine cascade is initiated with the secretion of corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) from the hypothalamus. This initiates the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which in turn triggers the secretion of glucocorticoids (i.e. cortisol in humans) from the adrenal glands. The glucocorticoids then provide feedback to inhibit subsequent CRH and ACTH secretion to regulate the HPA axis. The glucocorticoid cascade hypothesis postulates that the loss of glucocorticoid binding receptors (i.e. the glucocorticoid receptor and the mineralocorticoid receptor) in the hippocampus due to aging or degenerative processes leads to negative feedback insensitivity and subsequent glucocorticoid hypersecretion, and prolonged exposure to elevated

levels of glucocorticoids can lead to the atrophy of neuronal and dendritic processes, inhibition of neurogenesis and neuroplasticity, and neuronal cell death, resulting in further hippocampal damage (Sapolsky, 2000; Sapolsky et al., 1986).

Depression has been frequently linked to dysregulation of the HPA axis; studies consistently demonstrate increased cortisol levels in depressed individuals, with stronger effects observed in older adults. The association between depression and ACTH levels is less clear, and there is no evidence to indicate CRH levels are altered in depression (Belvederi Murri et al., 2014; Stetler & Miller, 2011). The relationship between depression and HPA axis activity may be more complex, as several studies observed decreased cortisol levels in depressed older adults instead (Bremmer et al., 2007; Morrison et al., 2000; Oldehinkel et al., 2001; Penninx et al., 2007). These findings provide some evidence that both hyper- and hypoactivity of the HPA axis are associated with LLD, which may reflect distinct depression subtypes and underlying aetiological pathways (Bremmer et al., 2007; Penninx et al., 2007). It has been hypothesised that such hypocortisolaemia common in many stress-related disorders including post-traumatic stress disorder, chronic fatigue syndrome, and fibromyalgia may reflect exhaustion of the HPA axis after a prolonged period of hyperactivity due to chronic stress (Heim et al., 2000). However, as most of the studies examining the HPA axis-depression association are cross-sectional in nature, causal inferences cannot be drawn, and it remains unclear whether HPA dysregulation is the cause or the effect of depression.

2.4.4 The neurotrophic hypothesis

Neurotrophic factors are critical in the regulation of neuronal and synaptic plasticity, and play a role in in the maintenance of neuronal functions and neurogenesis. It has been proposed that decreased expression of neurotrophic and growth factors leads to neuronal atrophy and decreased neurogenesis in limbic brain structures that are implicated in mood regulation, and may contribute to the pathophysiology of depression (Duman & Li, 2012). BDNF is one of the major neurotrophins, and animal models have demonstrated downregulation of BDNF expression in the hippocampus following exposure to stress, particularly in the dentate gyrus as well as the CA1 and CA3 subfields (Duman & Monteggia, 2006). Few studies have examined the

BDNF levels in older adults and the results are not always consistent. Significantly decreased serum and plasma BDNF levels have been reported in depressed older adults in a number of studies, including LOD (Shi et al., 2010), subthreshold depression (Chu et al., 2012), non-medicated LLD (Diniz et al., 2010) as well as those in remission (Laske et al., 2010), while another study reported no association with LOD (Dalby et al., 2013).

Other neurotrophic or growth factors may also be involved in LLD. For instance, reductions in serum glial cell-line derived neurotrophic factor have been observed in older adults with MDD compared to age- and sex-matched controls (Diniz et al., 2012; Tseng et al., 2013), but another study reported increased plasma glial cell-line derived neurotrophic factor levels in older adults with LOD (Wang et al., 2011). Serum nerve growth factor was also significantly reduced in depressed older adults, although levels were similar between the currently depressed and the remitted groups, suggesting that serum nerve growth factor levels may be a trait marker of LLD (Diniz et al., 2013b). The relationship between serum insulin-like growth factor 1 and LLD is less clear, with some evidence to suggest potential interactions between sex, level of insulin-like growth factor 1 concentrations and severity of depression (Emeny et al., 2014; van Varsseveld et al., 2015). Plasma vascular endothelial growth factor was found to be positively associated with depressive symptoms both at baseline and at 12 months, but was not among the top analytes useful for discrimination between older adults with and without depressive symptoms (Arnold et al., 2012).

2.4.5 The inflammatory hypothesis

Inflammation is a complex adaptive response of the body to injuries or pathogens. In recent years, there has been increasing evidence to suggest that inflammation and cell-mediated immune activation play important roles in the pathophysiology of depression (Maes, 2011; Miller et al., 2009a). This is particularly relevant to LLD, as many genes regulating inflammatory processes are upregulated and peripheral immune responses are increased in late life, leading to chronic neuroinflammation and excessive release of proinflammatory cytokines. It has been proposed that neuroinflammatory changes in ageing and ageing-related diseases may contribute

to part of the pathophysiology of depressive syndromes in late life (Alexopoulos & Morimoto, 2011).

Studies examining blood inflammatory markers have repeatedly shown that there is increased inflammation in MDD, and in particular LLD, though findings have not always been consistent. Among all the markers investigated, elevated interleukin (IL)-6 appears to be the most consistently associated with LLD (Baune et al., 2012; Bremmer et al., 2008; Dentino et al., 1999; Forti et al., 2010; Kern et al., 2014; Martínez-Cengotitabengoa et al., 2016; Penninx et al., 2003; Tiemeier et al., 2003). Conflicting results have been found for tumour necrosis factor (TNF)-α (Baune et al., 2012; Forti et al., 2010; Martínez-Cengotitabengoa et al., 2016; Penninx et al., 2003; van den Biggelaar et al., 2007) and cell adhesion molecules (ICAM-1 and VCAM-1) (Baune et al., 2012; Dimopoulos et al., 2006; Forti et al., 2010; Lee et al., 2009; Thomas et al., 2007). Limited evidence have been found for other inflammatory markers or cytokines as only a few studies have examined them in relation to LLD, namely α_1-antichymotripsin (Forti et al., 2010), IL-1α (Lee et al., 2009), IL-1β (Thomas et al., 2005; van den Biggelaar et al., 2007), IL-1ra (Martínez-Cengotitabengoa et al., 2016; Milaneschi et al., 2009; van den Biggelaar et al., 2007); IL-8 (Baune et al., 2012; Kern et al., 2014; Martínez-Cengotitabengoa et al., 2016), neutrophil gelatinase-associated lipocalin (Naudé et al., 2013), and plasminogen activator inhibitor-1 (Baune et al., 2012). Most studies that measured C-reactive protein (CRP) and IL-10 reported no association (Baune et al., 2012; Forti et al., 2010; Kop et al., 2002; Stewart et al., 2009; Tiemeier et al., 2003). A recent study using a data-driven, machine learning approach to identify a peripheral proteomic panel associated with LLD showed a range of proteins related to immuno-inflammatory processes were differentially expressed in LLD, including neutrophil gelatinase-associated lipocalin, IL-23, immunoglobulin E, cluster of differentiation 40 antigen, and IL-12p40 (Diniz et al., 2016).

While studies of LLD have primarily focused on inflammatory cytokines, other immune markers such as chemokines are potentially relevant as well. Chemokines are a family of proteins that are produced with the activity of chemotactic cytokines, and they have been shown to play a role in the regulation of a range of neuromodulation, neuroendocrine and neurotransmitter functions

(Stuart et al., 2015). However, only the aforementioned machine learning proteomic study appears to have included chemokines in its investigation, and the human CC chemokine-4 was among one of the significant circulating biomarkers identified (Diniz et al., 2016).

2.4.6 Potential role of the microbiota-gut-brain axis

More recently, there have been suggestions that the microbiota-gut-brain axis may mediate these processes underlying neuropsychiatric disorders including depression. It is now increasingly recognised that there is bidirectional communication between the gastrointestinal tract and the central nervous system via several pathways, including the autonomic nervous system (the enteric nervous system and the vagus), the neuroendocrine system, and the immune system (Cryan & Dinan, 2012). While the evidence is largely limited to preclinical studies to date, they have provided some support that alterations in the gut microbiota may be linked to depression-like behaviours and the associated neurobiological disturbances. For example, germ-free mice showed exaggerated HPA axis response to stress and reduced cortical and/or hippocampal BDNF and GR expression levels relative to specific pathogen free mice, and the exaggerated HPA axis response was fully reversed by reconstitution with the probiotic *Bifidobacterium infantis* and partially reversed by reconstitution with faecal matter from the specific pathogen free mice at an early developmental stage (Sudo et al., 2004). Microbiota may also influence serotonergic neurotransmission as studies have shown male germ-free mice showed elevated hippocampal levels of 5-HT and 5-hydroxyindoleacetic acid compared to control animals (Clarke et al., 2013).

In humans, administration of a probiotic formulation of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 for 30 days was associated with reductions in the global severity index and somatisation, depression and anger-hostility subscale scores of the Hopkins Symptom Checklist as well as the global score and anxiety subscale score of the Hospital Anxiety and Depression Scale in healthy adult volunteers (Messaoudi et al., 2010). Another small study showed correlations between faecal microbiota (a proxy for gut microbiota) and Montgomery-Åsberg Depression Rating Scale (MADRS) scores in a small non-geriatric sample (Naseribafrouei et al., 2014). However, more clinical studies need to be conducted to elucidate the role of the microbiota-gut-brain axis in depression, as well as specifically in LLD.

2.4.7 Cross talk between multiple systems

None of these hypotheses stated above, in and of themselves, sufficiently explain the pathogenesis of LLD, and it is likely that alterations in a number of the aforementioned systems interact with each other to contribute to the development of LLD. Firstly, inflammatory cytokines have stimulatory effects on HPA axis hormones and CRH in the hypothalamus and amygdala, which increases the production and release of ACTH and glucocorticoids, and the increased glucocorticoids downregulates the immuno-inflammatory response to prevent overactivity, forming an immunomodulatory feedback circuit. However, proinflammatory cytokines can also lead to glucocorticoid resistance through reduced GR function and expression, and eventually chronic inflammation in a feed-forward cascade (Haroon et al., 2012).

Secondly, inflammatory cytokines affect monoamine synthesis and metabolism. Proinflammatory cytokines can induce an enzyme called indoleamine 2,3-dioxygenase, which catabolises tryptophan into tryptophan catabolites including kynurenine rather than serotonin, leading to serotonin depletion. Moreover, kynurenine can be further metabolised into quinolinic acid in microglia which in excess can lead to excitotoxicity, or kynurenic acid in astrocytes which has downstream effects on the release of dopamine. Inflammatory cytokines may also reduce levels of tetrahydrobiopterin, a cofactor for enzymes involved in the synthesis of 5-HT, dopamine and noradrenaline, resulting in disruptions in monoaminergic neurotransmission (Haroon et al., 2012). Furthermore, another pathway that may be affected is the mitogen-activated protein kinase (MAPK) pathway. Animal models show that cytokines such as TNF-α and IL-1β leads to a time- and dose-dependent increase in the expression and activity of the serotonin transporter (Zhu et al., 2006; Zhu et al., 2005). MAPK pathways also influence functioning of the dopamine transporter, with activation of MAPK increasing dopamine reuptake in cell lines, and treatment of rat striatal synaptosomes with MAPK inhibitors decreasing dopamine reuptake in a time- and dose-dependent manner (Morón et al., 2003).

There is also a reciprocal relationship between the 5-HT system and the HPA axis, as serotonin has excitatory effects on the HPA system and modulates the release of CRH and AVP, while glucocorticoids regulate 5-HT synthesis and receptor function (Chaouloff, 1993). Early studies

showed that the association between cortisol and 5-HT turnover is dose-dependent, with increased 5-HT turnover observed following administration of a low dose of corticosterone and decreased 5-HT turnover following a high dose in rats (Kovács et al., 1977a; Kovács et al., 1977b). More recent studies found that the relationship is also time-dependent, with chronic exposure to corticosterone leading to decreased binding at 5-HT_{1A} and 5-HT_{1B} receptors (Mendelson & McEwen, 1992a, 1992b) as well as reduced 5-HT_{1A} receptor messenger ribonucleic acid (mRNA) expression (Meijer & de Kloet, 1994).

The pathways mentioned above appear to be closely entwined, but since depression is a highly heterogeneous disorder, there may still be some clinical benefits in delineating different depression subtypes as there is some evidence to suggest different depression subtypes or symptoms may reflect disruptions in specific systems. In a study examining the relationship between a range of inflammatory and metabolic variables and depression in a non-geriatric sample, melancholic depression was associated with HPA axis dysfunction whereas atypical depression was associated with inflammatory and metabolic dysregulation (Lamers et al., 2013). Moreover, when older adults in a large community-based study were assigned to 'vascular', 'degenerative' and 'inflammatory' subgroups based on their medical history, the results showed that the different risk-indicators are associated with distinct clinical profiles; the presence of a vascular risk-indicator was predicted by loss of energy, psychomotor change and sleep disturbance, the degenerative risk-indicator was predicted by psychomotor change and thinking/concentration disturbance, and the inflammatory risk-indicator was predicted by appetite disturbance, loss of energy, thoughts of death, sleep disturbance and thinking/concentration disturbance (Naarding et al., 2005).

2.5 Summary and gaps in the current literature

Depression, including subthreshold depressive symptoms, is not uncommon in late life. It presents a huge public health problem as it is associated with poor prognosis and frequent comorbidity with a wide range of chronic medical conditions, leading to increased health care costs.

There is currently a prolific body of literature on the structural and functional neurobiological alterations associated with LLD. Evidence from neuroimaging studies highlight that there are both grey matter and white matter abnormalities in LLD. These abnormalities are predominantly observed in fronto-limbic and fronto-striatal circuits, yet it is uncertain whether they represent the cause or effect of the disorder. While it is generally accepted that genetics play an important role in determining an individual's susceptibility to LLD, few candidate gene studies have been conducted to date. The few candidate gene studies have had limited success in identifying susceptibility polymorphisms, which may largely be due to inadequate statistical power to detect small genetic effects. A number of environmental risk factors as well as GxE interactions have also been identified, but more research needs to be conducted to replicate these findings. The role of epigenetics in LLD should also be explored further.

Based on findings from clinical, neuroimaging, neuropathology and genetic studies, several theories on the pathophysiology of LLD have been postulated. They suggest abnormalities in the monoaminergic neurotransmitter system, HPA axis regulation, neurotrophins system, immuno-inflammatory response regulation, and potentially microbiota-gut-brain axis regulation may contribute to LLD. A high burden of vascular disease may also underlie LLD.

This review laid the foundation for the research in this thesis and highlighted the areas in which research is lacking and/or inconsistent. This thesis aims to address these gaps in the literature by examining genetic, environmental and epigenetic factors contributing to LLD using data from two cohort studies of older Australians. Integration of several lines of evidence is expected to extend current findings on risk factors and the pathophysiology of LLD, and potentially shed light on the neurobiological pathways and processes involved in the pathogenesis of LLD.

3 METHODS

This chapter describes the cohort studies from which the samples used in this thesis were drawn, the measures used as dependent or independent variables as well as covariates used in the analyses, and finally the study designs and statistical methods used.

3.1 Study samples

Study samples in this thesis are subsamples of the Older Australian Twins Study (OATS) and the Sydney Memory and Ageing Study (MAS).

3.1.1 The Older Australian Twins Study (OATS)

OATS is a longitudinal cohort study of older twins that was initiated in 2006, with the primary objective of investigating genetic and environmental contributions to healthy brain ageing and age-related neurocognitive disorders.

Older twins in the three eastern states of Australia (New South Wales, Victoria and Queensland) were recruited via Twins Research Australia as well as a recruitment drive including newspaper advertisements and media campaigns. The inclusion criteria were: aged 65 years or over, an ability to consent, having a consenting co-twin, having completed some education in English, and being at least of low average IQ (IQ ≥ 80, as estimated from the National Adult Reading Test (Nelson & Willison, 1991)). Exclusion criteria were a diagnosis of a malignancy or other life-threatening medical illness, inadequate English to complete a neuropsychological assessment, and/or a current diagnosis of an acute psychotic disorder. All participants provided written informed consent. The study was approved by the ethics committees of Twins Research Australia, Melbourne Health, Queensland Institute of Medical Research and the South Eastern Sydney and Illawarra Area Health Service.

The baseline sample was comprised of 623 individuals – 160 monozygotic (MZ) and 125 dizygotic (DZ) twin pairs, 1 MZ triplets, and 52 singletons (29 single twins and 23 siblings of twins) (see Figure 3.1 for the OATS flowchart). Participants received a comprehensive assessment at baseline and were followed up every two years, and co-twins were interviewed

separately but temporally close to each other. The assessment protocol is standardised across the three study centres. Data collected include clinical, psychiatric, neuropsychological, cardiovascular, metabolic, biochemical, neuroimaging, genomic and proteomic measures. Zygosity was determined based on self-report and deoxyribonucleic acid (DNA) markers. The subsamples used in this thesis were comprised of twin pairs. The demographics of all twin pairs at baseline are presented in Table 3.1. Further details of the study design and methodology have been reported elsewhere (Sachdev et al., 2009).

Table 3.1. Demographics of twin pairs in OATS at baseline (*M* ± *SD* or *n* (%)).

	MZ (*n* = 160 pairs)	DZ (*n* = 125 pairs)
Age at baseline, years	70.7 ± 5.7	70.6 ± 5.2
Sex, female	204 (63.8)	171 (68.4)
Education, years	11.1 ± 3.3	11.4 ± 3.4
Genetic background		
- Caucasian	318 (99.4)	250 (100)
- Pacific Islander	2 (0.6)	0 (0)
Adjusted MMSE	28.5 ± 1.8	28.7 ± 1.4

Abbreviation: MMSE – Mini-Mental State Examination

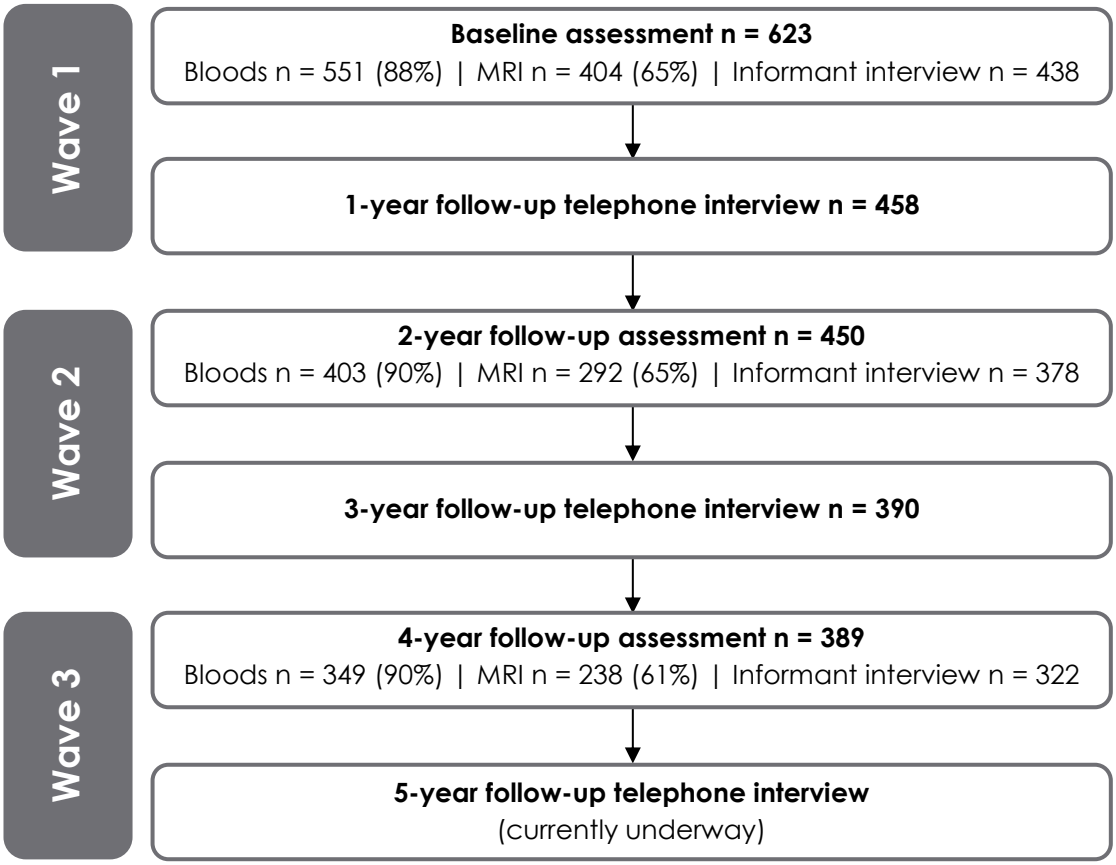

Figure 3.1. OATS flowchart.

3.1.2 The Sydney Memory and Ageing Study (Sydney MAS)

Sydney MAS is a longitudinal cohort study of older adults. Initiated in 2005, the aims of the study include examining the clinical characteristics and prevalence of mild cognitive impairment (MCI) and related syndromes in non-demented older Australians, determining the rate of cognitive decline over time, identifying predictors of cognitive decline and dementia, as well as assessing behavioural and psychological symptoms in MCI.

Participants were randomly recruited from two federal electorates of Sydney, New South Wales, Australia (Kingsford-Smith and Wentworth). Registration on the electoral roll is compulsory in Australia. Inclusion criteria were: aged 70 to 90 years, living in the community, and an ability to consent. Exclusion criteria were: inadequate English for neuropsychological testing, a diagnosis of dementia or a Mini-Mental State Examination (MMSE) (Folstein et al., 1975) score of less than 24 adjusted for age, education and non-English speaking background (NESB) (Anderson et

al., 2007) at baseline, psychotic symptoms, schizophrenia or bipolar disorder, multiple sclerosis, motor neurone disease or central nervous system inflammation, developmental disability, progressive malignancy or other conditions that may have interfered with the completion of assessments. All participants provided written informed consent. The study was approved by the ethics committees of the University of New South Wales and the South Eastern Sydney and Illawarra Area Health Service.

The study protocol and the assessments that participants received were similar to OATS. The baseline sample included 1037 participants (see Figure 3.2 for the Sydney MAS flowchart). The sample demographics at baseline are presented in Table 3.2. Further details of the study have been published elsewhere (Sachdev et al., 2010; Tsang et al., 2013).

Table 3.2. MAS sample demographics at baseline.

	M ± *SD* or *n* (%)
Age at baseline, years	78.8 ± 4.8
Sex, female	572 (55.2)
Education, years	11.6 ± 3.5
Genetic background	
- Caucasian	1016 (98.0)
- Asian	10 (1.0)
- Mixed	3 (0.3)
- Other	4 (0.4)
Adjusted MMSE	28.7 ± 1.3

Abbreviation: MMSE – Mini-Mental State Examination

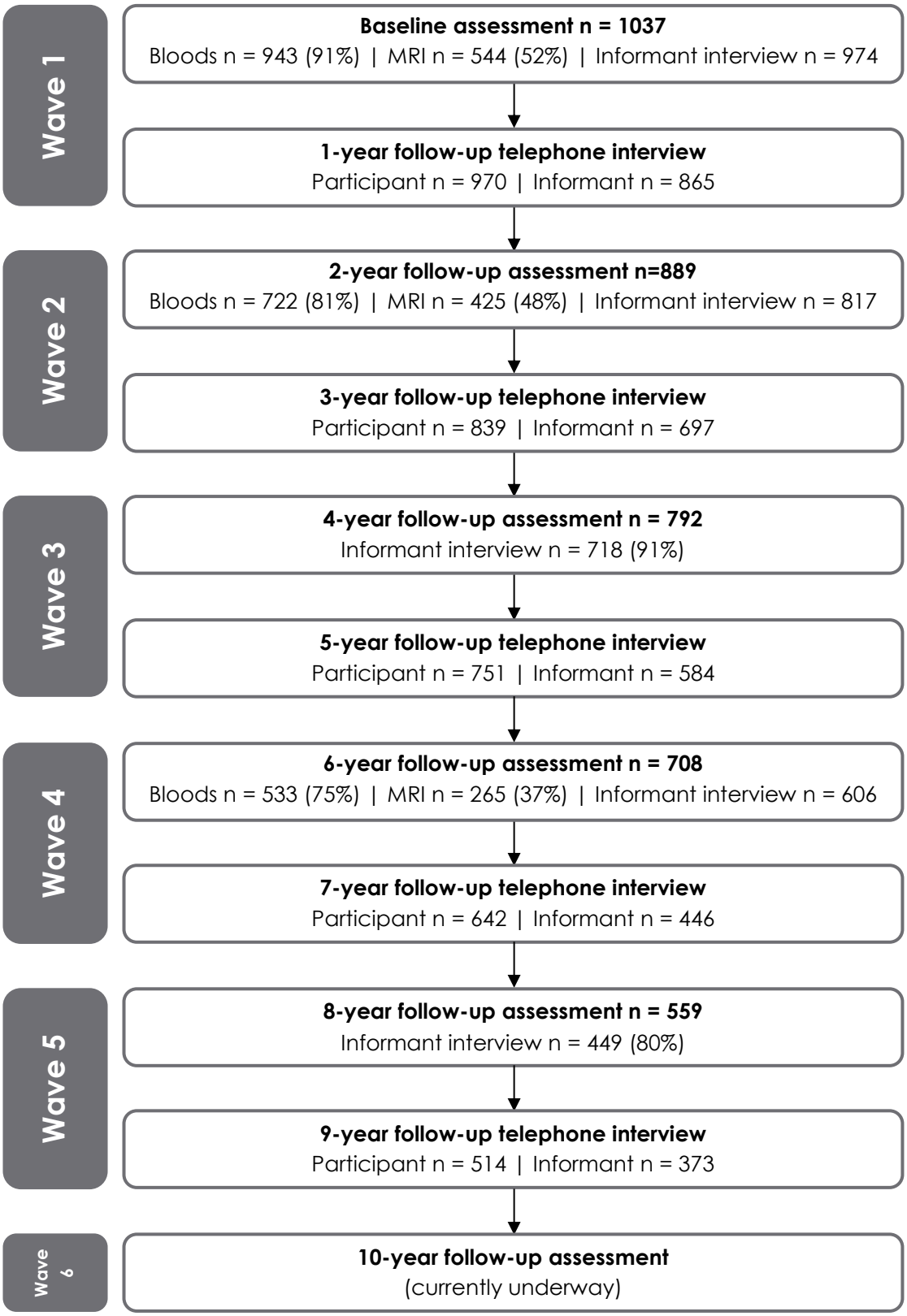

Figure 3.2. MAS flowchart.

At each assessment wave in OATS and Sydney MAS, participants completed a comprehensive battery of neuropsychological tests (see Table 3.3 below) and informants completed the Bayer Activities of Daily Living questionnaire (Hindmarch et al., 1998) in the telephone interview.

Table 3.3. Neuropsychological tests used to form domain scores in OATS and Sydney MAS.

Cognitive domain	Neuropsychological test	Normative data source and adjustments
Attention / processing speed	Digit Symbol (Wechsler, 1997a)	Age (Wechsler, 1997a)
	Trail Making Test A (Strauss et al., 2006)	Age and education (Tombaugh, 2004)
Memory	Logical Memory Story A delayed recall (Wechsler, 1997b)	Education (Grundman et al., 2004)
	Rey Auditory Verbal Learning Test (Strauss et al., 2006)	Age (Ivnik et al., 1992a; Ivnik et al., 1990)
	RAVLT total learning (sum of trials 1-5)	
	RAVLT short-term delayed recall (trial 6)	
	RAVLT long-term delayed recall (trial 7)	
	Benton Visual Retention Test recognition (Benton et al., 1996)	Age and education (Lechevallier-Michel et al., 2004)
Language	Boston Naming Test – 30 items (Kaplan et al., 2001)	Age (Fastenau et al., 1998)
	Semantic fluency (animals) (Strauss et al., 2006)	Age and education (Tombaugh et al., 1999)
Visuospatial	Block Design (Wechsler, 1981)	Age (Ivnik et al., 1992b)
Executive function	Controlled Oral Word Association Test (FAS) (Strauss et al., 2006)	Age and education (Tombaugh et al., 1999)
	Trail Making Test B (Strauss et al., 2006)	Age and education (Tombaugh, 2004)

OATS and Sydney MAS participants with neuropsychological or functional profiles indicating a possibility of dementia (neuropsychological performance at least 1.5 standard deviations below published norms in at least one cognitive domain and/or informant-rated Bayer-ADL adjusted total score of at least 3.0) were presented at a case conference. Consensus diagnoses were made by an expert panel of clinicians including neuropsychiatrists, psychogeriatricians and clinical neuropsychologists using available data.

Dementia/major neurocognitive disorder was diagnosed based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV and DSM-5) criteria (American Psychiatric Association, 2000, 2013). NESB participants were classified as cognitively normal if they performed within the normal range, and as having dementia if they received a consensus diagnosis of dementia due to the questionable validity of applying norms derived from individuals of English-speaking background (Kochan et al., 2010). Such cognitive classifications have been used as part of the exclusion criteria in the studies, with individuals classified as having dementia excluded from most analyses.

As allele frequencies and genotype distributions are known to differ between groups of different genetic background (Risch et al., 2002; Tang et al., 1998; Verhagen et al., 2008), only Caucasians were included in the studies in this thesis.

3.2 Measures

3.2.1 Psychosocial measures

3.2.1.1 Depression

The primary outcome of interest is late-life depression (LLD). Depressive symptoms were measured using the 15-item Geriatric Depression Scale (GDS-15) (Yesavage & Sheikh, 1986). The GDS-15 is a self-report depression screening scale for older adults, and is scored by counting the number of responses that indicate depression. Total scores range from zero to 15, with higher scores corresponding to a greater number of depressive symptoms, and a score of six or above indicating clinically significant depressive symptoms (Herrmann et al., 1996). GDS-15 total scores were considered invalid if 20% or more items were missing, or were pro-rated if there were fewer than 20% items missing. By applying a cut-off of six or above, the GDS-15 has a sensitivity of 85% and a specificity of 74% in elderly outpatients aged over 65 years when compared with the validated interviewer-rated Montgomery-Åsberg Depression Rating Scale (MADRS) (Herrmann et al., 1996).

In both OATS and Sydney MAS we used the GDS-15 with item 9 as described in Brink (1982; here item 12). There is high agreement between classifications based on the original version and

the version used in OATS and Sydney MAS ($\kappa = 0.93$). The internal consistency for the GDS-15 in both samples was fair (Cronbach's $\alpha = 0.74$ in Wave 1 of OATS, $\alpha = 0.73$ in Wave 1 of Sydney MAS and $\alpha = 0.77$ in Wave 4 of Sydney MAS). A total of 30 participants from Wave 1 of OATS (5% of valid data), as well as 73 participants from Wave 1 (7% of valid data) and 80 participants from Wave 4 of Sydney MAS (12% of valid data) scored six or above.

Participants also provided information on the medications they were taking in a self-report questionnaire. Based on the assumption that individuals on antidepressants are likely to report fewer or subthreshold symptoms with successful treatment and may be overlooked, participants were classified as being depressed if they scored six or above on the GDS-15 or if they self-reported using antidepressants. This definition of depression was used across all empirical studies in this thesis.

3.2.1.2 Anxiety

Anxiety was assessed using the nine-item anxiety subscale from the Goldberg Depression and Anxiety Scales, often known as the Goldberg Anxiety Scale (GAS) (Goldberg et al., 1988). The GAS is scored by summing the number of items endorsed. If none of the first four items were endorsed, the latter five items would not be administered, making it a simple screening tool that can be used in general medical settings. Total scores range from zero to nine, with higher scores indicating more anxiety symptoms, and a score of five or above suggesting clinically significant anxiety symptoms. Applying the 4/5 cut-off, the GAS has been shown to have a sensitivity of 82% compared with diagnoses made according to DSM-III criteria (Goldberg et al., 1988). The GAS demonstrated good internal consistency in Wave 1 of OATS (Cronbach's $\alpha = 0.82$).

3.2.1.3 Childhood maltreatment

In Wave 4 of Sydney MAS, early-life adversity was measured using the Childhood Trauma Questionnaire-Short Form (CTQ-SF), a 28-item retrospective self-report questionnaire designed to screen for childhood maltreatment. The CTQ-SF has 25 clinical items to assess histories of abuse or neglect, and a three-item minimisation/denial scale to detect underreporting of such experiences. All items are scored on a 5-point Likert-type scale, with item responses categories coded as 1 = 'never true', 2 = 'rarely true', 3 = 'sometimes true', 4 = 'often true', and 5 = 'very often

true'. The CTQ-SF has a five-factor structure, namely emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect (Bernstein et al., 2003); other traumatic events that may happen during childhood such as death of a family member or major illness are not assessed. The CTQ-SF also includes a 3-item Minimization/Denial scale to detect potential underreporting of maltreatment experiences.

Individuals can be classified as having none, low, moderate or severe trauma based on their subscale scores (see Table 3.4 below) as per Bernstein and Fink (1998).

Table 3.4. Classification of Childhood Trauma Questionnaire-Short Form subscale scores according to Bernstein & Fink (1998).

	None	Low	Moderate	Severe
Emotional abuse	5-8	9-12	13-15	16-25
Physical abuse	5-7	8-9	10-12	13-25
Sexual abuse	5	6-7	8-12	13-25
Emotional neglect	5-9	10-14	15-17	18-25
Physical neglect	5-7	8-9	10-12	13-25

Alternatively, Walker et al. (1999) used receiver operating characteristic curves to obtain cut-off scores for the subscales, and found that cut-off scores of 9/10 for emotional abuse, 7/8 for physical abuse, 7/8 for sexual abuse, 14/15 for emotional neglect, and 9/10 for physical neglect achieved sensitivities and specificities of 0.85 or better. This dichotomous classification was used for the analyses in Chapter 6.

The CTQ-SF has been validated in heterogeneous samples, including adult substance-dependent inpatients at drug and alcohol rehabilitation units, adolescent psychiatric inpatients, adult substance abusers in the community, and a normative community sample of adults (Bernstein et al., 2003). The authors observed satisfactory internal consistency in the subscales – alphas of 0.84-0.89 for emotional abuse, 0.81-0.86 for physical abuse, 0.92-0.85 for sexual abuse, 0.85-0.91 for emotional neglect, and 0.61-0.78 for physical neglect – as well as good criterion validity, with all subscale scores significantly correlated with therapist ratings in a subsample of adolescent psychiatric inpatients for whom corroborative data were available.

Internal consistency observed in the Sydney MAS sample, measured using Cronbach's α, are as follow: 0.67 for the overall scale, 0.77 for emotional abuse, 0.66 for physical abuse, 0.84 for sexual abuse, 0.88 for emotional neglect, and 0.51 for physical neglect.

3.2.1.4 Holocaust trauma

Aside from the CTQ-SF, participants also completed a self-reported questionnaire that assessed various types of stressful early life events including birth problems, family dysfunction, natural disaster, and war events. One of these questions concerns experience of Holocaust trauma. The actual wording used in that item was "Were you a Holocaust victim?" with the definition of a Holocaust victim provided for reference being "a person who lived in a country at the time when it was under Nazi regime, under Nazi occupation, or under the regime of Nazi collaborators or who fled a country or region not under Nazi rule or occupation due to Nazi rule or Nazi occupation." Responses were coded as 0 = 'No', 1 = 'Yes' and 888 = 'Don't know'.

3.2.2 Biological measures

3.2.2.1 Vascular diseases and risk factors

In both OATS and Sydney MAS, participants completed a medical history interview at each biannual assessment, which included questions about vascular diseases and risk factors (e.g., stroke, transient ischaemic attack, diabetes, hypertension, hypercholesterolaemia, dyslipidaemia, angina, and atrial fibrillation). Participants also completed a medical examination at each assessment wave, in which sitting systolic and diastolic blood pressure were measured. Hypertension was defined as previous diagnosis by a physician and current treatment for hypertension, or current systolic blood pressure ≥ 160mmHg, or diastolic blood pressure ≥ 95mmHg.

3.2.2.2 Genotyping

DNA was extracted from peripheral blood leukocytes using standard procedures in Wave 1 of the Sydney MAS. *APOE* genotyping was carried out using Taqman assays (Applied Biosystems Inc., Foster City, CA, USA) and genome-wide genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix Inc., Santa Clara, CA, USA). After quality

control procedures as described in Mather et al. (2016), genome-wide genotyping data was available for 925 Sydney MAS participants.

3.2.2.3 DNA methylation

DNA was extracted from peripheral blood leukocytes using standard procedures in Wave 1 of OATS. DNA methylation status was measured using the Illumina Infinium HumanMethylation450 BeadChip (450K) (Illumina Inc., San Diego, CA, USA) in 110 MZ twin pairs. The 450K array interrogates methylation levels in 485,764 cytosine positions across the human genome. An analysis of the distribution of the probes revealed that 30.9% of them were located in CpG islands, 23.1% in shores (i.e. 2kb from CpG islands), 9.7% in shelves (i.e. 2-4kb from CpG islands), and the remaining 36.3% in others or 'open sea' regions (Sandoval et al., 2011). Sex chromosome probes, probes containing single-nucleotide polymorphisms (SNPs), cross-reactive probes as well as probes not detected in all samples were excluded from the analysis (Chen et al., 2013a). After quality control procedures there were 420,982 out of 485,764 probes remaining, with 32.3% in CpG islands, 23.5% in shores, 9.3% in shelves, and the remaining 34.9% in others or 'open sea' regions.

3.2.3 Covariates

A range of covariates were considered in the analyses. Age and sex were typically entered as covariates since the literature commonly reports a female preponderance in depression and an increased risk with age (Cole & Dendukuri, 2003; Schoevers et al., 2000). Smoker status was coded as 0 = 'non-smoker', 1 = 'past smoker', and 2 = 'current smoker', with the reference period being the past month. Drinker status was coded as 0 = 'abstainer', 1 = 'past drinker', and 2 = 'current drinker', with the reference period being the past year. Physical activity was a continuous variable of total minutes of physical activity per week, weighted per mild, moderate and vigorous types of physical activity.

3.3 Study designs and statistical methods

3.3.1 Statistical packages

Raw data were stored and managed in IBM SPSS Statistics Version 24 (IBM Corp., 2016). IBM SPSS Statistics Version 24 (IBM Corp., 2016) was used for descriptive data analyses in all studies as well as logistic regression in Chapter 6, Review Manager (RevMan) Version 5.3 (The Nordic Cochrane Centre, 2014) was used for meta-analyses in Chapter 5, and R (R Core Team, 2014) was used for multivariate twin modelling in Chapter 4 as well as the DNA methylation study in Chapter 7.

3.3.2 Descriptive data analyses

Group differences in demographic and clinical characteristics were explored using independent *t*-tests for continuous variables and χ^2-tests for categorical variables. For continuous variables with a non-normal distribution, group differences were tested with the non-parametric Mann-Whitney test. Test statistics are reported with the corresponding degrees of freedom (*df*) and the two-tailed *p*-value. Bivariate correlations were computed using Pearson's *r*. Internal consistency of various scales used was computed using Cronbach's α. All statistical tests were two-tailed at the $\alpha = 0.05$ level of statistical significance unless otherwise specified.

3.3.3 Missing data

Total scores on rating scales are pro-rated if there were less than or equal to 20% missing data. More than 20% missing data on any rating scale was treated as invalid and those participants were excluded from the analyses.

3.3.4 Heritability and genetic correlations

The classical twin design is a widely used design that compares the phenotypic resemblances of MZ and DZ twins to explain the sources of phenotypic variation. The two main assumptions of the classical twin design are (i) MZ twins are genetically identical and DZ twins share half of their segregating genes, and (ii) shared environmental influences are approximately the same for MZ and DZ twins reared in the same family.

The observed phenotypic variation (V_P) can be partitioned into genetic and environmental components. The genetic component can be further decomposed into additive genetic effects (A) and non-additive genetic effects (often denoted D for dominant), and the environmental component can be further decomposed into common or shared environmental effects (C) and unique or non-shared environmental effects (E). The E component also includes measurement error. Hence, the phenotypic covariance of MZ twins is estimated to be $\sigma^2_A + \sigma^2_D + \sigma^2_C$ and the phenotypic covariance of DZ twins is estimated to be $0.5\sigma^2_A + 0.25\sigma^2_D + \sigma^2_C$. Since the C and D components cannot be modelled simultaneously with data limited to twins reared together, generally C is modelled where there is greater evidence for common environmental effects (i.e. when $r_{MZ} < 2r_{DZ}$), and D is modelled if there is greater evidence for non-additive genetic effects (i.e. when $r_{MZ} > 2r_{DZ}$) (van Dongen et al., 2012). Narrow-sense heritability (h^2) refers to the proportion of phenotypic variation that is due to additive genetic effects: $h^2 = V_A / V_P$ and can be modelled using structural equation modelling. Figure 3.3 shows an example of a univariate twin model.

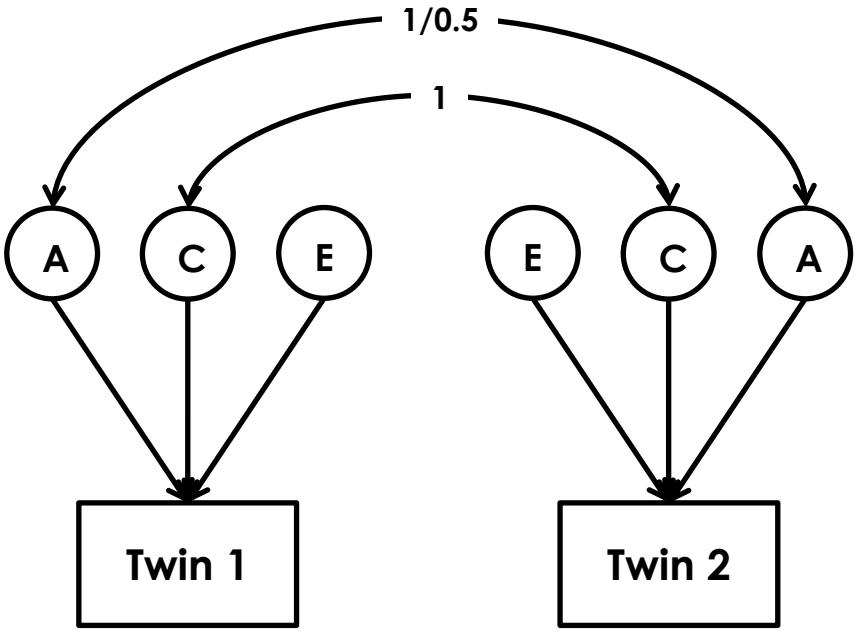

Figure 3.3. Example of a univariate ACE model. A – additive genetic effects, C – shared environmental effects, E – unique environmental effects. The additive genetic correlation is 1.0 for MZ twins and 0.5 for DZ twins.

Cross-twin-cross-trait covariances can be modelled in multivariate twin models, which allow the relative contributions of genetic and environmental influences to phenotypic covariation to be examined. Using multivariate twin models, we can then estimate the degree to which phenotypic covariations is due to overlapping genetic influences (genetic correlation, r_A), shared environmental influences (shared environmental correlation, r_C), or non-shared environmental influences (non-shared environmental correlation, r_E). These correlations are independent of the magnitude of the genetic and environmental contributions on each phenotype.

Cholesky decomposition is the most commonly used multivariate technique in the Classical Twin design. It is a method of triangular decomposition, where the first latent factor (η_1) explains variance in the first as well as all remaining variables (y_1, …, y_n), and the second latent factor (η_2) explains variance in the second as well as all remaining variables (y_2, …, y_n), and so on until the final latent variable (η_n) explains the final variable in the model (y_n) (see Figure 3.4) (Gillespie & Martin, 2005).

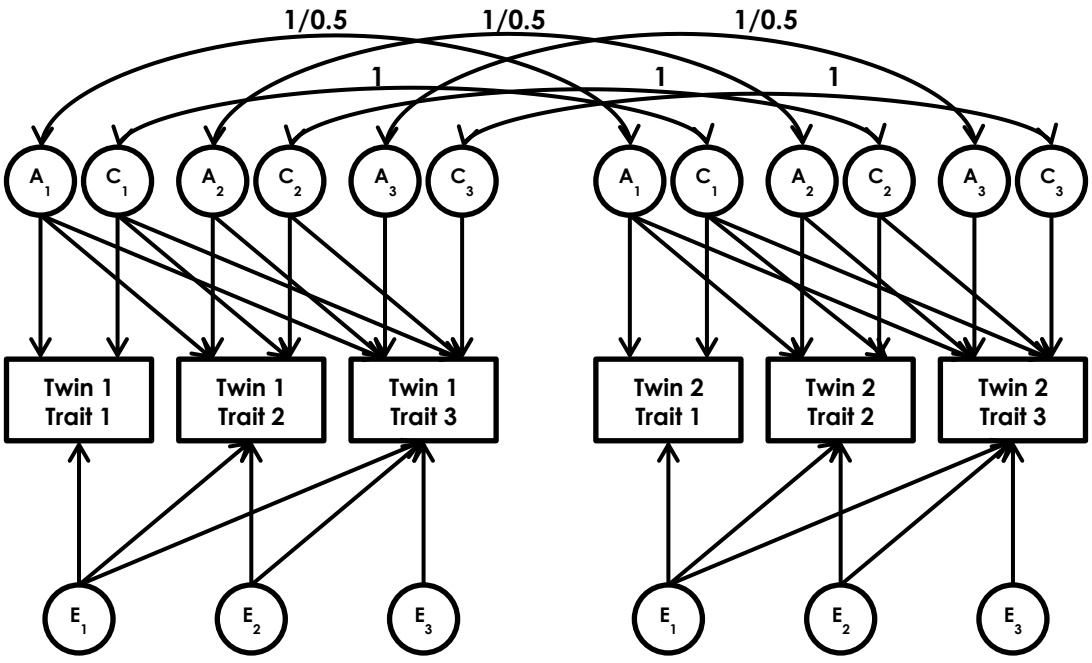

Figure 3.4. An example of a multivariate model of 3 traits using Cholesky decomposition. A – additive genetic effects, C – shared environmental effects, E – unique environmental effects. The additive genetic correlation is 1.0 for MZ twins and 0.5 for DZ twins.

Univariate and multivariate twin models can be modelled using OpenMx (Boker et al., 2016; Neale et al., 2016), a statistical package for structural equation modelling and matrix algebra optimisation that runs in R (R Core Team, 2014). OpenMx uses maximum likelihood estimation to estimate model parameters. In modelling dichotomous trait twin data, a liability threshold model is used, which assumes that the liability to develop the trait of interest has an underlying continuous normal distribution, and the discrete phenotype emerges once the liability exceeds a certain threshold (Falconer & Mackay, 1996).

A multivariate twin model with Cholesky decomposition was used to model genetic and environmental influences on LLD, anxiety, and hypertension in a subsample of OATS participants in Chapter 4. The multivariate twin modelling was carried out Dr Anbupalam Thalmuthu at the Centre for Healthy Brain Ageing, University of New South Wales, Australia.

3.3.5 Systematic review and meta-analysis of genetic association studies

Population-based genetic association studies seek to evaluate the association between a specific genetic polymorphism and the risk of a certain disease. In the current paradigm of genetic epidemiology, the common disease-common variant hypothesis states that complex disease can largely be attributed to a moderate number of common variants, each of which explaining a modest but significant risk. Such small effects require large samples to detect them, and genetic association studies are often statistically underpowered to do so. Synthesising evidence from multiple small studies is a potential solution to this problem.

In a meta-analysis, effect size estimates of multiple small studies are pooled, with the contribution of each study weighted according to study size, based on the assumption that larger studies are likely to give more accurate effect size estimates. Two models can be used to pool the effect size estimates from various studies – the fixed effects model (Mantel-Haenszel) or the random effects model (Simonian and Laird). The fixed effects model assumes that study samples are drawn from a single population, and therefore the differences in observed effect size estimates across studies are only due to sampling error, whereas the random effects model

assumes that study samples can be drawn from different populations, and both sampling error and between-study heterogeneity contribute to the observed differences. A meta-analysis also allows the degree and source of between-study heterogeneity as well as potential publication bias to be investigated. Subgroup analyses may also be conducted to find out whether other study characteristics mediated the associations.

In Chapter 5, following a systematic search of LLD genetic association studies, meta-analyses were conducted for polymorphisms that have been examined in three or more independent published studies, with effect sizes estimated using pooled odds ratios (*OR*s) and 95% confidence intervals (95% CI). Between-study heterogeneity were tested and quantified. Potential biases including the winner's curse phenomenon and publication bias were also investigated.

3.3.6 Genetic, environmental and gene-environment predictors

Given that the primary outcome measure (depression) in this thesis is dichotomous, binary logistic regression was used to investigate genetic, environmental and gene-environment risk factors in LLD:

$$log\frac{\pi}{1-\pi} = \beta_0 + \beta_1 X_1 + \cdots + \beta_i X_i$$

where $\pi = \frac{e^{(\beta_0 + \beta_1 X_1 + \cdots + \beta_i X_i)}}{1+e^{(\beta_0 + \beta_1 X_1 + \cdots + \beta_i X_i)}}$ represents the probability for an individual being depressed, β_1, β_2, …, β_i are the coefficients of the predictor variables, and X_1, X_2, …, X_i are the predictor variables.

Prior to examining genetic associations between candidate polymorphisms and LLD as well as potential gene-by-environment interactions, potential deviations from Hardy-Weinberg equilibrium (HWE) were tested in the non-depressed group using the Pearson's goodness-of-fit test, as deviations from HWE may indicate genotyping errors or population stratification. The assumption of logistic regression that the relationship(s) between any continuous predictor(s) and the logit of the dependent variable was tested using the Box-Tidwell test.

In Chapter 5, the role of candidate polymorphisms that have been identified in meta-analyses as significantly associated with LLD or with MDD were investigated using Wave 1 data from the Sydney MAS cohort. Separate regression models were conducted for each polymorphism, with depression status as the dependent variable, and genotype as the independent variable. Genotypes were coded as 0 = 'common homozygote', 1 = 'heterozygote', and 2 = 'rare homozygote' as the genetic model is unknown.

In Chapter 6, the effects of early-life trauma were investigated using Wave 4 data from the Sydney MAS cohort. The impact of five types of childhood maltreatment assessed using the CTQ-SF as well as Holocaust trauma on LLD was investigated in two separate logistic regression models, with depression status as the dependent variable, and all five CTQ-SF childhood maltreatment variables as independent variable in one model, and the Holocaust trauma variable as independent variable in the other model.

To explore potential gene-by-environment interactions, a childhood maltreatment composite variable was created based on the five CTQ-SF variables, coded as 0 = 'no history of maltreatment' and 1 = 'positive history of maltreatment'. Each binary logistic regression had depression status as the dependent variable, one of the candidate polymorphisms investigated in Chapter 5, one of the early-life trauma variables (the childhood maltreatment composite variable or Holocaust trauma variable), as well as an interaction term between the candidate polymorphism and early-life trauma as independent variables.

3.3.7 DNA methylation changes

The discordant MZ twin design, also known as the co-twin control design, represents a powerful design to assess disease-associated biomarkers, as it offers the advantage of controlling for a range of potential confounding factors such as genetic background, age, sex, maternal influences, early environmental experiences to some extent as well as population cohort effects (Bell & Spector, 2011; van Dongen et al., 2012).

In Chapter 7, an epigenome-wide association study (EWAS) was conducted using the beta values on each probe as the dependent variable, and depression status as the independent variable.

After regressing out the first two principle components of white blood cell counts and the first four principle components of beta values to adjust for cell composition and batch effects respectively, associations between DNA methylation levels and LLD were investigated using the entire MZ twin sample in the OATS cohort with available DNA methylation data. Associations between mean differences in DNA methylation levels between cases and controls and LLD were tested using generalised estimating equations to account for the twin structure, with age, sex, smoking status, drinking status and level of physical activity included as covariates. An alternate analysis was undertaken using independent *t*-tests in the subset of MZ twin pairs discordant for depression, as this provides some degree of controlling for potential confounding factors such as genetic background, age, sex, maternal influences, early environmental experiences to some extent as well as population cohort effects (Bell & Spector, 2011; van Dongen et al., 2012). The Benjamini-Hochberg correction was used to account for multiple testing. The EWAS was carried out by Dr Nicola Armstrong at Murdoch University, Australia.

4 GENETIC AND ENVIRONMENTAL INFLUENCES ON LATE-LIFE DEPRESSION AND ITS COVARIATION WITH ASSOCIATED PHENOTYPES

4.1 Introduction

As reviewed in the previous chapter, depression is a neuropsychiatric disorder that commonly occurs across the lifespan, and genetic factors are believed to play a significant role in its aetiology. The genetics of depression in adulthood has been extensively investigated, providing consistent evidence for its familial aggregation. The heritability of major depressive disorder (MDD) in adulthood has been estimated at 37%, with minimal effect of shared environment and substantial effect of unique environment in a meta-analysis of five twin studies (Sullivan et al., 2000). In contrast, in late life to date, there have only been five published studies examining the heritability of depression or depressive symptoms, two of which involved overlapping samples (see Table 4.1 for a summary of the studies). Most of these studies reported that late-life depression (LLD) or depressive symptoms are moderately heritable (Carmelli et al., 2000; Jansson et al., 2004; Johnson et al., 2002; McGue & Christensen, 1997). For the studies that also computed sex-specific heritability estimates, the heritability estimates for LLD or depressive symptoms were generally higher for women than for men, but none of these studies found statistically significant sex differences (Jansson et al., 2004; Johnson et al., 2002; McGue & Christensen, 1997). In contrast, conflicting findings have been reported with regards to whether the heritability of LLD or depressive symptoms changes with age. Increasing heritability with age is a recognised phenomenon in behavioural genetics and has been observed for a range of phenotypes between adolescence to young adulthood (Bergen et al., 2007) as well as in late life (Steves et al., 2012). One of the main explanations is active gene-environment correlation; as individuals age they become more independent and seek out environmental exposures as a function of their genetic predispositions. Three studies have examined this, with one cross-sectional and one longitudinal study reporting that the heritability increases with age (Carmelli et al., 2000; Gatz et al., 1992); however, the remaining cross-sectional study reported no significant age differences in heritability (Johnson et al., 2002). Since heritability estimates are population-specific, methodological differences such as sample characteristics, how the

phenotype was defined and whether analyses were stratified by age or sex could have contributed to these discrepancies.

Table 4.1. Summary of published heritability studies of LLD or depressive symptoms.

Study	Sample	Instrument	Best-fitting/most parsimonious model	Heritability estimates	Findings
Gatz et al. (1992)	▪ Swedish Adoption/Twin Study of Aging (SATSA) ▪ 68 MZ and 161 DZ twin pairs reared apart, 114 MZ and 138 DZ twin pairs reared together ▪ 62% female ▪ Separated into two cohorts: old (≥60 years, mean age=70 years) vs young (<60 years, mean age=48 years)	CES-D	The full model fitted for the total scale and all three subscales in the full sample. When stratified by age, the additive genetics parameter was important only for the psychomotor retardation subscale in the old cohort.	▪ Total scale: 16% Depressed Mood subscale: 7% ▪ Psychomotor Retardation subscale: 19% ▪ Well-Being subscale: 0% ▪ Stratified by age: old – 18%, young – 3% ▪ The heritability is significantly higher in old cohort ($p<.01$).	Late-life depressive symptoms are only modestly heritable. Genetic influences are important only for psychomotor retardation and somatic complaints in the old cohort; whereas environmental influences explain entirely the variance in the other subscales.
McGue & Christensen (1997)	▪ Longitudinal Study of Aging Danish Twins (LSADT) ▪ 115 female MZ, 173 female DZ, 52 male MZ, 66 DZ twin pairs ▪ 71% female ▪ Mean age approximately 80 years	CAMDEX depression section plus an additional 11 items	Both the AE and DE models fitted the data well, with the DE model fitting better than the AE model; results of the AE model are reported here as it is practically unlikely to have significant genetic dominance effects in the absence of additive genetic effects.	▪ Total scale: 27% ▪ Affect subscale: 16% ▪ Somatic subscale: 20% ▪ Stratified by sex: o Total scale: female – 33%, male – 16% o Affect subscale: female – 18%, male – 11% o Somatic subscale: female – 25%, male – 12%	LLD symptoms are moderately heritable. No significant sex differences in heritability were observed. Common genetic effects contribute substantially to the phenotypic correlation between subscales.

Study	Sample	Instrument	Best-fitting/most parsimonious model	Heritability estimates	Findings
Carmelli et al. (2000)	▪ National Heart, Lung and Blood Institute (NHLBI) Twin Study ▪ 83 MZ and 84 DZ white, male, veteran twin pairs ▪ Mean age=62.8 years at baseline	CES-D	AE model	▪ Baseline: 25% ▪ 10-year follow-up: 55% ▪ The heritability at follow-up is significantly higher than that at baseline (p<0.01).	The heritability of depressive symptoms increases with age. The stability in depressive symptoms is mainly due to the continuity of genetic influences.
Johnson et al. (2002)	▪ Study of Middle-Aged Danish Twins (MADT) and the Longitudinal Study of Aging Danish Twins (LSADT) ▪ Includes the full sample used in McGue & Christensen (1997) ▪ 562 female MZ, 640 female DZ, 471 male MZ, 496 male DZ twin pairs ▪ Aged ≥45 years	CAMDEX depression section plus an additional 11 items	AE model with relative constraints on age and sex	▪ Total scale: 29% ▪ Affect subscale: 27% ▪ Somatic subscale: 26%	Depression is moderately heritable in older adults. No significant age differences in heritability were observed when the analysis was stratified into five age groups. No significant sex differences in heritability were observed. Common genetic effects explain a substantial proportion of the phenotypic correlation between the Affect and Somatic subscales.

Study	Sample	Instrument	Best-fitting/most parsimonious model	Heritability estimates	Findings
Jansson et al. (2004)	▪ OCTO-Twin Study, the Swedish Adoption/Twin Study of Aging (SATSA) and the Gender Study ▪ Unknown if sample overlaps with that of Gatz et al. (1992) ▪ 123 female MZ, 207 female DZ, 90 male MZ, 109 male DZ, 430 opposite-sex DZ twin pairs ▪ Mean age=72 years	Continuous depression score: CES-D Dichotomous depressed state: CES-D ≥ 16 or self-reported antidepressant use	Continuous measure: AE model with equal estimates for males and females, but the intraclass correlations suggest some sex differences. Dichotomous measure: AE models with equal or different estimates for males and females.	▪ Continuous measure: full sample – 23%, female – 30%, male – 16% ▪ Dichotomous measure: full sample – 33%, female – 49%, male – 9%	Both depressive symptoms and depressed state are moderately heritable in older women, but they are only modestly heritable in older men. The sex differences in heritability estimates, however, were not statistically significant.

Abbreviations: CAMDEX – Cambridge Mental Disorders of the Elderly Examination; CES-D – Centre for Epidemiologic Studies Depression Scale; DZ – dizygotic; MZ – monozygotic

As reviewed in Chapter 2, epidemiological and clinical studies have reported that LLD is frequently concomitant with psychiatric disorders and medical conditions. In particular, LLD often co-occurs with anxiety (Braam et al., 2014; Byers et al., 2010) as well as a range of vascular conditions including stroke (Li et al., 2015; Pan et al., 2011) and hypertension (Huang et al., 2010). Given that genetic factors are known to play an aetiological role in depression (Sullivan et al., 2000), anxiety (Hettema et al., 2001), stroke (Bevan et al., 2012) as well as hypertension (Gavras et al., 1999), it is of considerable interest to investigate whether shared genetic vulnerabilities may explain their co-occurrence. An early bivariate twin study examining the co-occurrence between depression and anxiety in a young adult twin sample (age = 30.1 ± 7.6 years) has reported a genetic correlation at unity (r_G = 1.00) (Kendler et al., 1992), a finding that has been subsequently replicated in a number of other adult cohorts (Kendler et al., 2006; Roy et al., 2009) but not in older adults. Depressive symptoms have also been shown to be genetically correlated with hypertension (r_G = 0.19) and heart disease (r_G = 0.42) in middle-aged male twins (age = 41.9 ± 2.7 years) (Scherrer et al., 2003), as well as with coronary artery disease (r_G = 0.16) in a slightly older twins cohort (mean age = 57 years) (Kendler et al., 2009). Yet despite the common comorbidity of depression and vascular disease and risk factors in late life, no study has yet explored the possible shared genetic influences on these phenotypes.

The aims of this chapter are to examine (i) the extent to which genetic, shared environmental and unique environmental influences contribute to the variation of LLD, and (ii) whether shared genetic influences explain the common co-occurrence between depression, anxiety, stroke and hypertension in late life.

4.2 Methods

4.2.1 Participants

The sample comprised participants from the Older Australian Twins Study (OATS; Sachdev et al., 2009). Monozygotic (MZ) and dizygotic (DZ) twin pairs from baseline (Wave 1) of OATS were included. The following were excluded: (i) participants of non-Caucasian ancestry, (ii) participants with missing data on any of the outcome variables included in the models and their

co-twins, and (iii) participants who had a consensus diagnosis of dementia or delirium or were cognitively unclassified and their co-twins.

4.2.2 Measures

Depressive symptoms were assessed using the 15-item short form of the Geriatric Depression Scale (GDS-15) (Yesavage & Sheikh, 1986). Its psychometric properties have been reported in Chapter 3. Higher scores on the GDS-15 correspond to more depressive symptoms, and a score of six or above indicates clinically significant depressive symptoms (Herrmann et al., 1996). The GDS-15 was treated as invalid if 20% or more items were missing, and the score was pro-rated if fewer than 20% of items were missing. Participants also provided information on their current medication use in a self-report questionnaire. As individuals on antidepressants are more likely to report fewer or subthreshold symptoms following successful treatment and may be overlooked, we classified participants as being depressed if they scored six or above on the GDS-15 or if they reported current antidepressant use.

Anxiety was assessed using the nine-item Goldberg Anxiety Scale (GAS) (Goldberg et al., 1988). Its psychometric properties have been reported in Chapter 3. Higher scores on the GAS indicate more anxiety symptoms and a score of five or above suggests clinically significant anxiety symptoms. The participants were also asked about their medical history during the interview, which included questions about their history of stroke and diagnosis of hypertension. Sitting systolic and diastolic blood pressure were measured during the assessment. Hypertension was defined as a composite variable based on previous diagnosis by a physician and current treatment for hypertension, or current systolic blood pressure ≥ 160mmHG, or diastolic blood pressure ≥ 95mmHg.

4.2.3 Statistical analyses

Differences between the zygosity groups were assessed using permutation tests. Twin similarity in MZ and DZ twins was assessed with the probandwise concordance rate, using the formula $\frac{2C}{(2C+D)}$ where C is the number of concordant twin pairs, and D is the number of discordant twin pairs (McGue, 1992; Smith, 1974). The probandwise concordance rate represents the risk that a

twin is affected if the co-twin is affected. Greater twin similarity in MZ twin pairs relative to DZ twin pairs indicates the contribution of genetic influences on a particular trait.

The total variance of each phenotype can be decomposed into three components, namely additive genetic effects (A), shared environmental effects (C) and unique environmental effects (E). It is presumed that MZ twins are genetically identical whereas DZ twins share half of their segregating genes, thus the expected twin covariances are $\sigma^2_A + \sigma^2_C$ for MZ twins and $0.5\sigma^2_A + \sigma^2_C$ for DZ twins. Multivariate genetic modelling was used to examine the heritability of LLD, anxiety, stroke and hypertension, as well as the genetic correlations between these phenotypes. Age and sex were included as covariates in all models as older age and female sex have been frequently shown to be associated with a higher risk of LLD or depressive symptoms in the literature (Beekman et al., 1995; Prince et al., 1998).

Models were fitted in OpenMx (Boker et al., 2016; Neale et al., 2016) using R version 3.1.0 (R Core Team, 2014). OpenMx uses maximum likelihood estimation and models dichotomous outcomes under a liability-threshold model, which assumes the liability to develop a disorder has an underlying continuous normal distribution, and the discrete phenotype emerges once the liability exceeds a certain threshold (Falconer & Mackay, 1996). A full Cholesky decomposition ACE model was fitted first, followed by an AE model, and the model fit was compared. The relative goodness-of-fit of the models was assessed using the minus two log-likelihood (-2LL) and Akaike's Information Criteria (AIC = $\chi^2 - 2df$). A lower -2LL value indicates better model fit and a lower AIC value indicates a more parsimonious model.

4.3 Results

A subsample of 130 MZ and 105 DZ twin pairs was selected from OATS for the present study. Sample characteristics and results from the comparisons of the two groups (MZ versus DZ) are presented in Table 4.2 below. No significant differences were observed on any of the outcome measures or covariates between the MZ and DZ groups.

Table 4.2. Characteristics of the sample used in this study (*M* ± *SD* or *n* (%)).

	MZ (*n* = 130 pairs)	DZ (*n* = 105 pairs)	Test statistic[a]	*p*
Age, years	70.1 ± 5.3	70.9 ± 5.3	−1.71	0.09
Sex, female	162 (62.3)	140 (66.7)	0.78	0.29
Depressed	37 (14.2)	29 (13.8)	0.02	0.90
Anxiety, GAS ≥ 5	24 (9.1)	19 (9.2)	0.01	0.90
Stroke	6 (2.3)	12 (5.7)	2.77	0.06
Hypertension	177 (68.1)	153 (72.9)	1.05	0.22

[a] Group differences were assessed using permutation tests (*t* for continuous variables, and Pearson's χ^2 for dichotomous variables).
Abbreviation: GAS – Goldberg Anxiety Scale.

4.3.1 Phenotypic concordance

For LLD, the probandwise concordance was 43.2% in MZ twins and 20.7% in DZ twins. For clinically significant anxiety symptoms, the probandwise concordance was 33.3% in MZ twins and 0% in DZ twins. For hypertension, the probandwise concordance was 83.6% in MZ twins and 75.8% in DZ twins. There were no twin pairs that were concordant for stroke.

4.3.2 Multivariate genetic modelling

As the prevalence of stroke was very low in this sample with no twin pairs concordant for stroke, it was excluded from the multivariate model. When both goodness-of-fit and parsimony were considered, the Cholesky AE model (−2LL = 1165.1, *df* = 1386, AIC = −1606.9) better explained the relationships between LLD, anxiety and hypertension compared to the Cholesky ACE model (−2LL = 1164.0, *df* = 1380, AIC = −1596.0) (see Figure 4.1 for a diagram of the AE model).

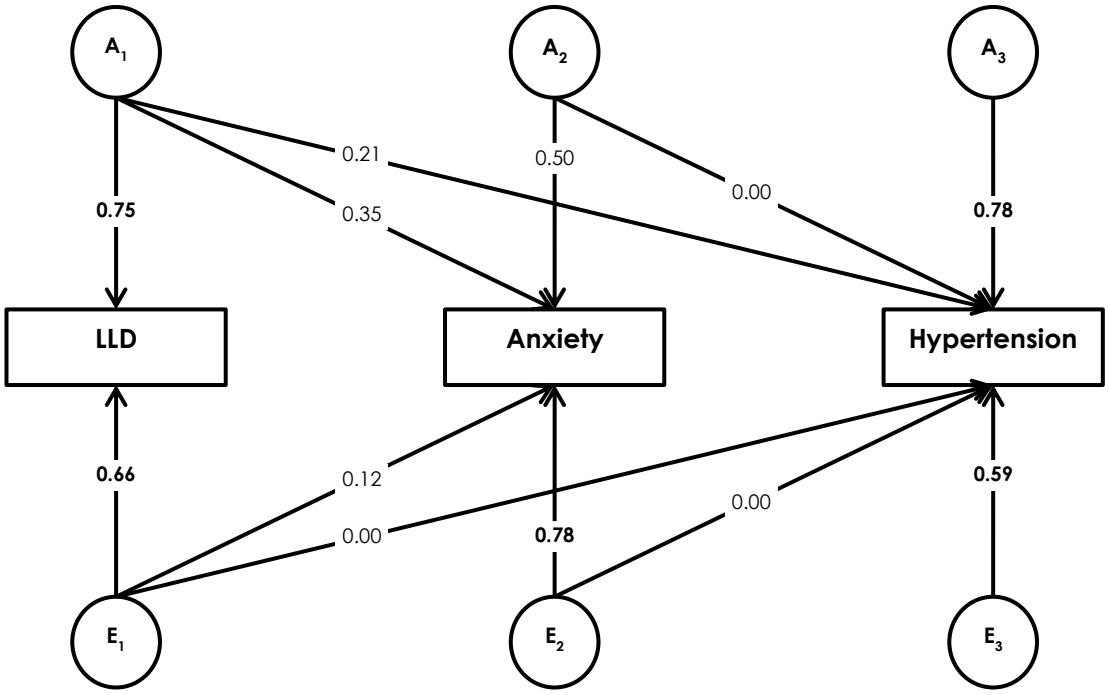

Figure 4.1. Path model with standardised path estimates for the best-fitting model for LLD, anxiety, and hypertension in older adults. Significant paths are indicated in bold.

All three phenotypes examined were significantly heritable; both LLD and anxiety were moderately heritable (h^2 = 0.57 and h^2 = 0.37 respectively), and hypertension was substantially heritable (h^2 = 0.65) (see Table 4.3). Genetic and environmental correlations between these phenotypes are presented in Table 4.4; significant positive genetic correlations were observed between depression and clinically significant anxiety symptoms (r_G = 0.58), as well as between clinically significant anxiety symptoms and hypertension (r_G = 0.15). None of the environmental correlations reached statistical significance.

Table 4.3. Heritability estimates for LLD, anxiety and hypertension.

Phenotype	Heritability estimate (h^2)
LLD	**0.57 (95% CI 0.27-0.79)**
Anxiety	**0.37 (95% CI 0.01-0.72)**
Hypertension	**0.65 (95% CI 0.43-0.80)**

Note: Significant results indicated in bold.

Table 4.4. Estimates of genetic and environmental correlations between LLD, anxiety and hypertension in older adult twins.

	Anxiety	Hypertension
Genetic correlation r_G (95% CI)		
LLD	**0.58 (0.05-1.00)**	0.25 (<0.00-0.59)
Anxiety		**0.15 (0.02-0.50)**
Environmental correlation r_E (95% CI)		
LLD	0.16 (<0.00-0.52)	0.00 (<0.00-0.00)
Anxiety		0.00 (<0.00-0.00)

Note: Covariates included in the model: age, sex. Significant results indicated in bold.

4.4 Discussion

In this chapter the genetic and environmental influences on LLD, anxiety and hypertension as well as the genetic and environmental correlations between these measures were investigated in a sample of older Australian twins. The results showed that both depression and anxiety are moderately heritable, whereas hypertension is substantially heritable in late life. Furthermore, we observed significant genetic correlations between depression and anxiety, as well as between anxiety and hypertension.

Previous twin studies reported a modest to moderate genetic influence on late-life depressive symptoms (ranging from 7% to 55% depending on how the phenotype was defined and whether the analysis was stratified by age or sex; see Table 4.1 above) (Carmelli et al., 2000; Gatz et al., 1992; Jansson et al., 2004; Johnson et al., 2002; McGue & Christensen, 1997). The only study that used a reasonably similar design and sample to the present study reported a heritability estimate of 33% for the dichotomous depressed state variable, which was defined as a composite based on the Centre for Epidemiological Studies – Depression Scale score combined with self-reported antidepressant use information, regardless of whether parameters were constrained to be equal in males and females (Jansson et al., 2004). The heritability estimate for LLD found in the present study lies towards the higher end of the range of all reported estimates, but as mentioned earlier, heritability estimates are population- and time-specific, and such variation in heritability estimates could have arisen from differences in demographic characteristics, genetic or environmental influences affecting specific populations, the criteria or instrument used to define the phenotype, or cohort effects (Bundey, 1991; Tenesa & Haley, 2013). The findings reported

here are, nonetheless, consistent with most prior studies that found shared environmental influences played minimal role in depression, whether in adulthood or specifically in late life (Carmelli et al., 2000; Jansson et al., 2004; Johnson et al., 2002; McGue & Christensen, 1997; Sullivan et al., 2000).

Depression and anxiety frequently co-occur throughout the lifespan, with between 14.5% and 35% of older adults with depression also meeting diagnostic criteria for anxiety (Lenze et al., 2000; Schoevers et al., 2003b; Schoevers et al., 2005); however, no previous study has sought to examine the source(s) of covariation between LLD and anxiety. In this sample of older twins, a moderate genetic correlation was observed between LLD and anxiety, which suggests shared genetic influences underlie the two phenotypes. This was not particularly surprising, as many have argued the two phenotypes have considerable symptom overlap and their 'comorbidity' is in fact an artefact of the current categorical nosology in psychiatry (Goldberg & Goodyer, 2005; Maj, 2005). Studies examining the biological correlates of depression and anxiety provide some evidence of common biological abnormalities underlying the two phenotypes, including dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Hek et al., 2013; Mantella et al., 2008; Penninx et al., 2007), as well as hypoactivity or altered functional connectivity in the prefrontal and orbitofrontal cortices (Aizenstein et al., 2009; Andreescu et al., 2015; Price et al., 2011) (see Chapters 2.2 and 2.4).

While vascular disease has been proposed to play an important role in the development of LLD (particularly the late-onset subtype), the few studies examining genetic correlations between depression and vascular phenotypes have used middle-aged cohorts. These studies generally reported modest but significant genetic correlations of depression with heart disease, hypertension and heart rate variability (r_G mainly in the range of -0.22 to 0.19) (Kendler et al., 2009; Scherrer et al., 2003; Su et al., 2012), with the only study reporting a much higher genetic correlation between depression and heart disease ($r_G = 0.42$) being one that used a sample substantially younger than the median age of onset of heart disease (Scherrer et al., 2003). The current study is the first to investigate the genetic correlation between depression and hypertension in older adults, but no significant genetic correlation was found. The moderate but

non-significant genetic correlation between depression and hypertension likely indicates inadequate statistical power, which potentially could be overcome by using a larger sample in the future.

In contrast, modest but significant shared genetic influences between anxiety and hypertension were observed in this study ($r_G = 0.15$). Epidemiological studies have previously reported associations between anxiety and cardiovascular disease and risk factors including hypertension (Carroll et al., 2010) and coronary heart disease (Kubzansky et al., 1998; Roest et al., 2010) in younger populations. Several plausible mechanisms have been proposed to explain the association between anxiety and cardiovascular disease and risk factors. One common explanation is the dysregulation of the HPA axis, which results in increased sensitivity to stress and chronic elevations of cortisol (Mantella et al., 2008; Wirtz et al., 2007). A second pathway that has been suggested to underlie the association between anxiety and cardiovascular disease and risk factors relates to oxidative stress and inflammation (Dinh et al., 2014; Hovatta et al., 2010; Ng et al., 2008). Variants in a number of genes have been reported to play a role in the regulation of these pathways, such as the glucocorticoid receptor (*NR3C1*) gene, the mineralocorticoid receptor (*NR3C2*) gene, the FK506 binding protein 5 (*FKBP5*) gene (DeRijk, 2009), and the apolipoprotein E gene (*APOE*) (Jofre-Monseny et al., 2008). Interestingly, these same biological mechanisms have also been implicated in depression (Belvederi Murri et al., 2014; Black et al., 2015; Palta et al., 2014); yet no significant genetic correlation was observed between depression and hypertension in this particular sample. Given the relatively small sample used in this study, further studies with larger samples are required to elucidate whether there are common genetic factors underlying depression and hypertension, as well as underlying LLD and stroke. Genetic correlations with other vascular phenotypes that may be more strongly associated with LLD, such as cerebral microvascular dysfunction (van Agtmaal et al., 2017), remain to be explored.

Several limitations need to be noted in the interpretation of these findings. This study is subject to the common limitations of the classical twin design, particularly the equal environment assumption (EEA). The validity of the EEA has drawn some debate, as a number of studies have

shown that MZ twins are exposed to more similar environments compared to DZ twins (also known as 'correlated environmental influences') (Bouchard & McGue, 2003; Joseph, 1998, 2002). The possibility of having correlated environmental influences challenges the assumption that genetic and environmental influences are independent, and would result in a biased heritability estimate due to model misspecification (Tenesa & Haley, 2013). However, two studies that have tested for the possible violation of the EEA in twin studies of psychiatric and substance use disorders found it tenable for these disorders (Kendler & Gardner, 1998; Kendler et al., 1993).

A further limitation of this study is that LLD was defined using a self-report questionnaire (GDS-15) and antidepressant use. While clinical diagnoses for depression were not available, previous psychometric studies have demonstrated that the GDS-15 has satisfactory sensitivity and specificity to detect depression in older adults across a range of settings (Almeida & Almeida, 1999; Friedman et al., 2005; Herrmann et al., 1996; Lesher & Berryhill, 1994). Moreover, subthreshold depressive symptoms are prevalent among older adults, and are undoubtedly clinically relevant as they are associated with similar detrimental outcomes as in MDD, including functional disability, cognitive impairment and higher mortality rates (Han et al., 2008; Lavretsky & Kumar, 2002; Lyness et al., 2006; Penninx et al., 1999).

As information on the age of onset of depressive symptoms was not available, the sample used in this study is likely a heterogeneous group that includes both early-onset recurrent depression and late-onset depression. The literature suggests that these two depression subtypes are associated with distinct clinical features, which may reflect aetiological differences. Potential differences in heritability of the early-onset and late-onset subtypes of LLD should be explored in the future. Potential sex differences were not examined in this study due to the small sample size; previous studies that tested for sex differences showed that the heritability of late-life depressive symptoms is slightly higher in females than in males, but the observed differences were not statistically significant (Jansson et al., 2004; Johnson et al., 2002; McGue & Christensen, 1997). It is, however, possible that the magnitude of shared genetic influences between the co-occurring phenotypes differ for males and females, as has been previously demonstrated in a younger adult sample (Burton et al., 2015).

This study adds to the limited literature examining the genetic and environmental influences on LLD or depressive symptoms and associated phenotypes, and sheds light on potential shared pathways that may be involved in their pathophysiology. More research is required to determine whether common genetic influences underlie the co-occurrence of depression and hypertension, and studies with sufficiently large samples are needed to investigate if behavioural risk factors mediate the genetic correlation as the association could reflect predisposition for behavioural risk factors such as smoking, excessive alcohol consumption and physical inactivity. Future studies examining genetic correlations between LLD and other vascular phenotypes may further elucidate the biological underpinnings of the depression-vascular disease comorbidity.

5 GENETIC ASSOCIATION STUDIES OF LATE-LIFE DEPRESSION

5.1 Introduction

The previous chapter examined the proportion of variance in late-life depression (LLD) that is accounted for by genetic and environmental influences respectively. It revealed that LLD is moderately heritable, with the remaining variance explained solely by unique environmental influences. This following chapter will explore the role of common candidate variants in relation to the susceptibility to develop LLD. The contents of this chapter have already been published (Tsang et al., 2017).

LLD is a complex neuropsychiatric disorder, which is believed to be caused by a myriad of genetic and environmental factors, with each factor conferring only a small effect on the aetiology of the disorder (Naismith et al., 2012). Despite the number of genetic association studies of LLD that have been conducted to date, there is limited evidence for robust susceptibility genes as findings across studies have been inconsistent. Such inconsistencies may arise from methodological differences including differences in the definition of depression and sampling strategies used, or from inadequate statistical power to detect modest genetic effects.

Meta-analysis is a potentially powerful statistical method to quantitatively synthesise results across multiple studies, and can potentially resolve the aforementioned discrepancies by pooling underpowered studies to detect associations and to identify sources of heterogeneity (Lohmueller et al., 2003). In recent years, comprehensive meta-analyses have been used to provide an overview of the molecular genetics of psychiatric disorders (López-León et al., 2008; Taylor, 2013; Warrier et al., 2015). While over 100 candidate gene studies of LLD have been published, only two meta-analyses of genetic association studies of LLD have been conducted, with each focusing on one particular candidate polymorphism. The first study examined the *BDNF* Val66Met polymorphism and reported a significant association between the Met allele and LLD (odds ratio (OR)$_{\text{Met vs Val/Val}}$ = 1.48, 95% confidence interval (CI) 1.13-193) (Pei et al., 2012), and the second one examined the *SLC6A4* 5-HTTLPR polymorphism and reported a

significant association between the short allele and LLD ($OR_{S\ vs\ L/L}$ = 1.29, 95% CI 1.01-1.66; $OR_{S/S\ vs\ L/L}$ = 1.68, 95% CI 1.20-2.35) (Gao et al., 2014).

The aim of this chapter is to conduct a systematic review and a meta-analysis of genetic association studies of LLD, including all polymorphisms that have been examined in the literature where possible. The candidate polymorphisms identified as significantly associated with LLD in this review as well as those associated with major depressive disorder (MDD) in an earlier meta-analysis (López-León et al., 2008) will then be examined in a subsample of the Sydney MAS.

5.2 Systematic review and meta-analysis

5.2.1 Methods

5.2.1.1 Search strategy

Studies were identified through a comprehensive, systematic search on MEDLINE, EMBASE and PsycINFO databases. The search strategy used based on keywords including "depress*", "mood disorder*", "affective disorder*", "older", "elder*", "geriatric", "late life", "late onset", "genetic*", "polymorphism*", "SNP*" and their corresponding subject headings in the respective databases (see Table A.1 in Appendix A for an example of the full search strategy used). Journal articles published in English before 18th February 2016 were considered. Moreover, reference lists of eligible studies and reviews as well as citation lists of eligible studies obtained through the Scopus database were hand-searched to identify additional potentially relevant studies.

5.2.1.2 Study selection

Case-control or cross-sectional studies that examined the association between one or more genetic polymorphism(s) and depression or depressive symptoms present in late life (using an age cut-off of 50 years or above), regardless of age of onset, were selected. The age cut-off of 50 years was chosen to be inclusive in capturing all potentially relevant studies. Disagreements on study inclusion were resolved through consensus. While the current literature suggests some differences in clinical, neuropsychological and neuroimaging characteristics between early-onset depression (EOD; typically with an onset in adolescence or early adulthood) recurrent in late life

and late-onset depression (LOD; first symptoms emerging in late life, studies have used various age cut-offs ranging from 50 to 65 years), which may reflect aetiological differences, the majority of genetic association studies of LLD do not differentiate between the two subtypes and the studies included in this review may include both.

Studies were included if case status was defined based on established psychiatric interviews or validated depression rating scales. Exclusion criteria were:

(1) the genotype distribution in the control group was not in Hardy-Weinberg equilibrium (HWE) (Munafò & Flint, 2004),
(2) the control group included individuals with a history of depression who at the time of assessment was in remission,
(3) the sample included individuals with other psychiatric or neurological disorders,
(4) there was insufficient data to estimate an effect size,
(5) the sample included individuals with psychotic depression, or
(6) studies of depression secondary to other medical conditions (e.g., post-stroke depression, depression in Alzheimer's disease etc.)

Where studies examining the same polymorphism involved potentially overlapping samples, the corresponding authors ($n = 5$) were emailed for further clarification. In situations where there was substantial sample overlap, only the study with the largest sample size was included.

5.2.1.3 Data extraction

The following information was extracted from eligible studies: first author's name, year of publication, country, ethnicity of sample, mean and standard deviation (or range) of sample age, percentage of females in the sample, diagnostic criteria or instrument used to assess depression, genotype frequencies for cases and controls or mean depression score for each genotype group, and source of sample. Data extracted was checked by Dr Simone Reppermund and Dr Karen Mather at the Centre for Healthy Brain Ageing, University of New South Wales, Australia. Corresponding authors ($n = 24$) were contacted for more information if sample characteristics or essential data required for the meta-analysis were missing from the published articles, three of whom could not be reached. In the end only five of them were able to provide the information or

data requested since many of the included studies were conducted more than a decade ago, and the corresponding authors no longer have access to the data.

5.2.1.4 Meta-analysis

Meta-analyses were conducted for polymorphisms that were investigated in at least three independent published studies. Pooled ORs and 95% CIs were estimated, and forest plots were obtained for each polymorphism. For studies reporting continuous depression rating scale scores, the standardised mean difference (SMD) was re-expressed as log OR using the following formula (Higgins & Green, 2011):

$$SMD = \frac{\sqrt{3}}{\pi} \ln OR$$

Between-study heterogeneity was tested using the Q-test (with $p < 0.10$ indicating significant heterogeneity), and the degree of inconsistency was quantified using the I^2 statistic. Pooled ORs were estimated using random effects models as they assume some degree of variability between study samples. Sensitivity analysis was performed by excluding the included study with the earliest publication date to test for the winner's curse phenomenon (Lohmueller et al., 2003). The winner's curse phenomenon refers to the tendency of overestimating genetic effect sizes in initial association studies, as the publication of these studies are conditional on the effects being statistically significant. Potential publication bias was evaluated using visual inspection of funnel plots for all meta-analyses that reached statistical significance. All statistical analyses were conducted using Review Manager (RevMan) Version 5.3 (The Nordic Cochrane Centre, 2014), with $p < 0.05$ (two-tailed) considered statistically significant.

5.2.2 Results

A total of 166 potentially relevant studies were identified for full-text review following a comprehensive search of the databases and a review of the titles and abstracts. After full-text review, 119 of them were excluded for reasons specified in the study selection flowchart (Figure 5.1), and 46 candidate gene studies and one genome-wide association study (GWAS) were included. The candidate gene studies examined polymorphisms in 23 genes and the functions of these genes are listed in Table 5.1. Only four out of the 23 candidate genes have been investigated in three or more independent studies, so meta-analysis was only performed on those four genes,

and the results are summarised in Table 5.2. Forest plots are presented in Figure 5.2. Detailed characteristics of studies included in each meta-analysis are presented in Tables A.2 to A.5 in Appendix A.

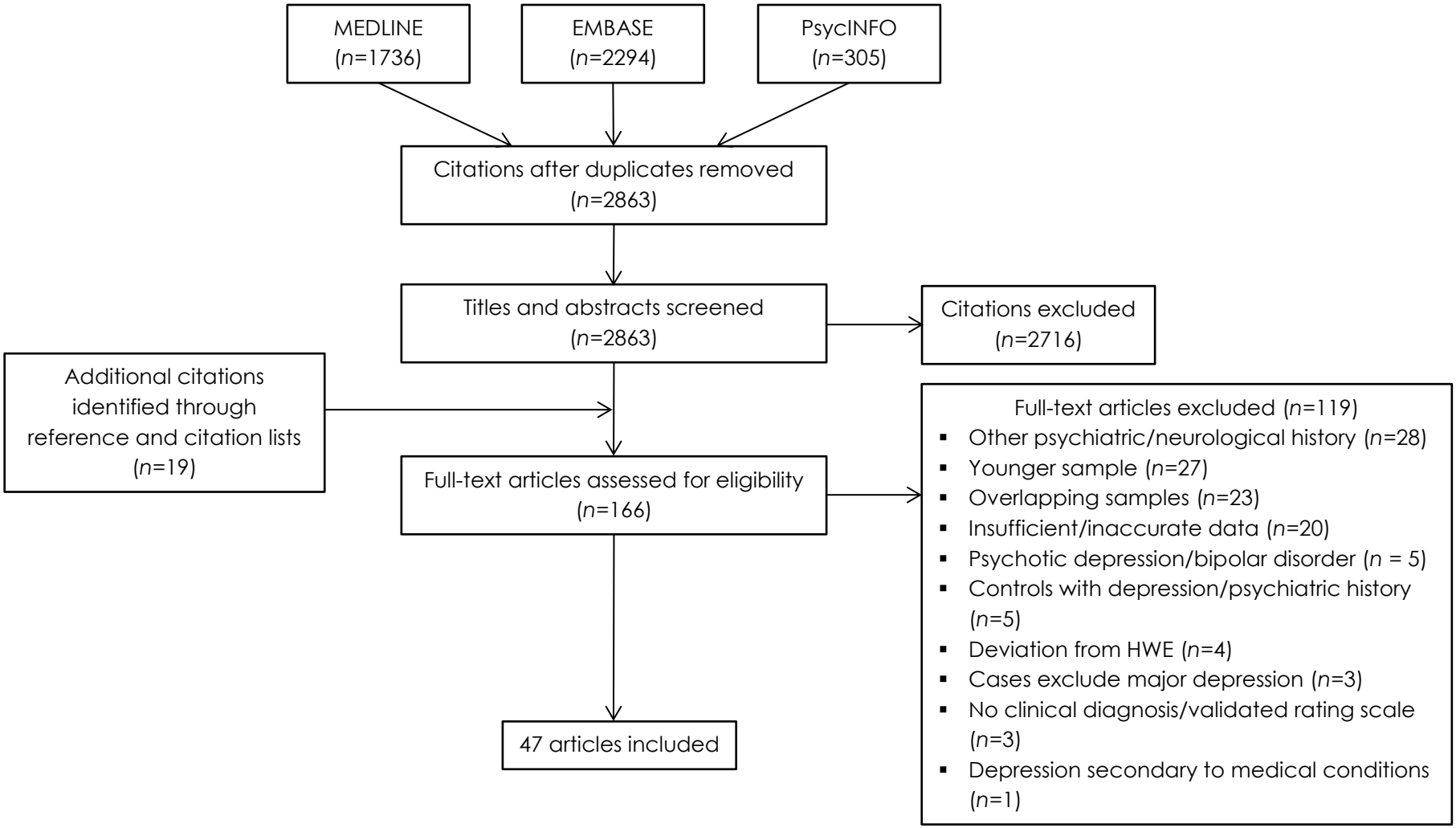

Figure 5.1. Study selection flowchart according to MOOSE guidelines (Stroup et al., 2000).

Table 5.1. Genes investigated in included studies and their functions.

Gene	Location	Functions
ACE	17q23	Regulates HPA axis activation and monoaminergic transmission
AGTR1	3q24	Regulates HPA axis activation and monoaminergic transmission
AKT1	14q32	Regulates neuronal survival and monoaminergic transmission
AKTIP	16q12	Modulates AKT1 activity
APOE	19q13	Involved in maintenance and repair of myelin and influences Aβ metabolism
BDNF	11p13	Regulates neuronal survival and neuroplasticity and mediates dopaminergic transmission
CNTF	11q12	Regulates neuronal survival and involved in injury responses
COMT	22q11	Mediates dopaminergic transmission
CRP	1q23	Mediates immune and injury responses
CRY1	12q23-q24	Regulates the circadian clock
CRY2	11p11	Regulates the circadian clock
IL1B	2q14	Mediates immune responses and HPA axis activation
IL10	1q31-q32	Mediates immune responses and HPA axis activation
LRP1	12q13	Regulates synaptic plasticity and influences Aβ metabolism
MTHFR	1p36	Involved in metabolism of homocysteine
NTRK2	9q22	Regulates neuronal survival, proliferation and differentiation
PCLO	7q21	Involved in the organisation of synaptic active zones and in monoaminergic transmission
PPARG	3p25	Regulates adipocyte differentiation and glucose homeostasis
SLC6A4	17q11	Mediates serotonin reuptake
TNF	6p21	Mediates immune responses and HPA axis activation
TPH2	12q21	Involved in biosynthesis of serotonin
TEF	22q13	Involved in circadian feedback loops
TFCP2	12q13	Mediates Aβ metabolism and tau phosphorylation

Abbreviations: Aβ – beta-amyloid; HPA – hypothalamic-pituitary-adrenal

Table 5.2. Results of meta-analyses of candidate gene studies of late-life depression.

Gene	Variant	Comparison	No. of studies	Test of association			Test of heterogeneity		
				OR	95% CI	*p*	*Q*	*p*	I^2
APOE	ε2/ε3/ε4	ε2/ε3 vs ε3/ε3	4	0.93	0.61-1.43	0.74	0.54	0.91	0%
		ε2+ vs ε2-	5	1.09	0.78-1.52	0.62	1.21	0.88	0%
		ε2 vs ε3	4	1.09	0.77-1.55	0.63	0.60	0.90	0%
		ε3/ε4 vs ε3/ε3	4	1.50	0.89-2.52	0.13	**6.48**	**0.09**	**54%**
		ε4/ε4 vs ε3/ε3	3	1.64	0.26-10.19	0.60	3.93	0.14	49%
		ε4+ vs ε4-	17	1.10	0.91-1.34	0.33	**29.4**	**0.02**	**46%**
		ε4 vs ε3	4	**1.49**	**1.03-2.17**	**0.04**	5.18	0.16	42%
BDNF	Val66Met	Val/Met vs Val/Val	4	1.05	0.69-1.61	0.82	5.50	0.14	45%
		Met/Met vs Val/Val	4	**1.72**	**1.10-2.69**	**0.02**	3.04	0.39	1%
		Met vs Val	4	**1.33**	**1.05-1.68**	**0.02**	3.76	0.29	20%
MTHFR	C677T	C/T vs C/C	5	1.15	0.66-2.02	0.62	**11.47**	**0.02**	**65%**
		T/T vs C/C	5	1.11	0.51-2.42	0.79	**8.03**	**0.09**	**50%**
		T vs C	4	1.41	0.76-2.61	0.28	**11.88**	**<0.01**	**75%**
SLC6A4	5-HTTLPR	S/L vs L/L	5	1.19	0.94-1.51	0.15	3.85	0.43	0%
		S/S vs L/L	5	**1.59**	**1.21-2.09**	**<0.01**	3.59	0.46	0%
		S vs L	4	**1.31**	**1.02-1.67**	**0.03**	4.38	0.22	32%

Note: Results in bold are statistically significant at $p<0.05$ for tests of association and at $p<0.10$ for tests of heterogeneity.

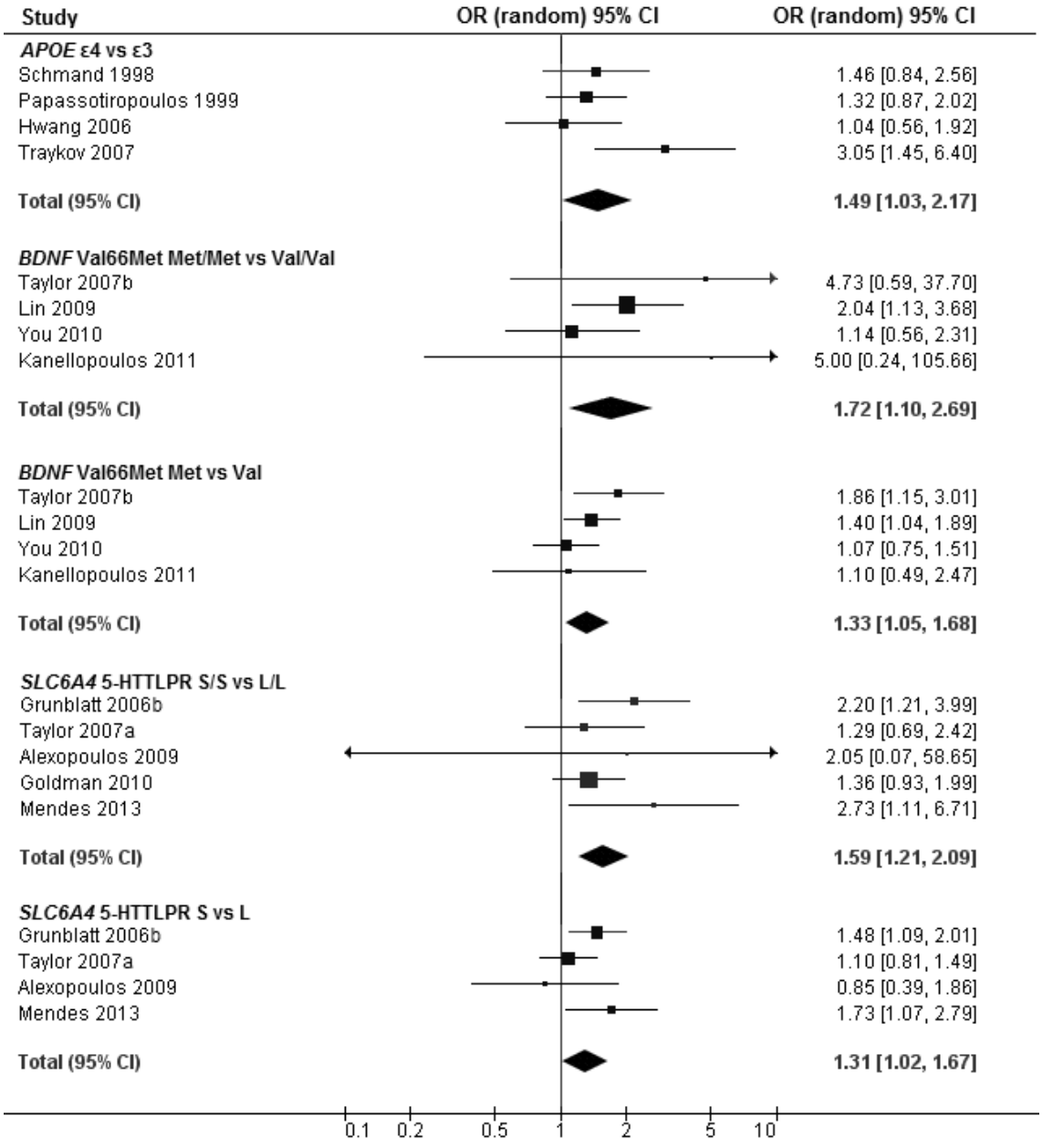

Figure 5.2. Forest plots of statistically significant meta-analyses.

5.2.2.1 APOE ε2/ε3/ε4 (rs429358 and rs7412)

The majority of the studies that investigated the *APOE* ε2/ε3/ε4 polymorphism reported data only on ε4 carriers versus non-carriers and HWE could not be assessed. For this reason, separate meta-analyses were conducted with (i) all of these studies included (Butters et al., 2003; Chen et al., 2013b; Class et al., 1997; Harwood et al., 1999; Hwang et al., 2006b; Nebes et al., 2001; Nose et al., 2013; Papassotiropoulos et al., 1999; Schahab et al., 2006; Schmand et al., 1998; Slifer et al., 2009; Sohrabi et al., 2009; Steffens et al., 2003a; Steffens et al., 2003b; Sureshkumar et al., 2012; Taylor et al., 2007a; Traykov et al., 2007), and (ii) only the studies that reported complete genotype frequencies with controls in HWE (Hwang et al., 2006b; Papassotiropoulos et al., 1999; Schmand et al., 1998; Traykov et al., 2007). Most of the studies were community-based and included mainly Caucasian participants, while the LLD phenotype was defined using a range of different instruments. When all the studies were pooled, there was significant between-study heterogeneity and the overall result was non-significant. Visual inspection of the forest plot suggested that the Traykov et al. (2007) study was the outlier, but omission of the study did not change the results. When only the four studies that reported complete genotype frequencies and were in HWE were pooled, there were a total of 366 cases and 610 controls with no significant between-study heterogeneity. The pooled OR for the ε4 allele compared to the ε3 allele was 1.49 (95% CI 1.03-2.17); none of the other comparisons reached statistical significance.

5.2.2.2 BDNF Val66Met (rs6265)

Four studies examined the *BDNF* Val66Met polymorphism in a total of 577 cases and 425 controls (Kanellopoulos et al., 2011; Lin et al., 2009; Taylor et al., 2007b; You et al., 2010). Two studies were hospital-based with Asian participants, but no significant between-study heterogeneity was observed. The pooled OR for the Met allele compared to the Val allele was 1.33 (95% CI 1.05-1.68) and the pooled OR for the Met/Met genotype compared to the Val/Val genotype was 1.72 (95% CI 1.10-2.69).

5.2.2.3 MTHFR C677T (rs1801133)

A total of five studies investigated the *MTHFR* C677T polymorphism, four of which were case-control studies with a total of 283 cases and 321 controls (Almeida et al., 2005; Chen et al., 2005;

El-Batch et al., 2010; Pan et al., 2009), and the remaining study reported mean depression scores by genotype in a further 323 participants (Lan et al., 2012). None of the comparisons reached statistical significance, and significant between-study heterogeneity was observed for all three comparisons tested (I^2 ranging from 50% to 75%). Two studies were hospital-based, and the majority of the samples were non-Caucasian. While the El-Batch et al. (2010) study appeared to be the main source of heterogeneity based on visual inspection of the forest plot, omission of the study did not change the results of the meta-analysis.

5.2.2.4 SLC6A4 5-HTTLPR (44bp Ins/Del)

The *SLC6A4* 5-HTTLPR polymorphism was investigated in five studies, four of which used a case-control design with a total of 406 cases and 688 controls (Alexopoulos et al., 2009; Grünblatt et al., 2006b; Mendes et al., 2013; Taylor et al., 2007a), and the remaining study reported mean depression scores by genotype in an additional 984 participants (Goldman et al., 2010). All included studies were community-based studies, and three of them used Caucasian samples. No significant heterogeneity was observed between these studies. When the results were pooled, the S allele was associated with a slightly higher risk of LLD (OR_{SvsL} = 1.31, 95% CI 1.02-1.67; $OR_{S/SvsL/L}$ = 1.59, 95% CI 1.21-2.09).

It should be noted that 5-HTTLPR alleles other than the commonly reported S and L alleles were observed in one of the five included studies. An additional extra-long (XL) allele was observed in Goldman et al.'s (2010) sample, and as the frequencies were relatively low (S/XL n = 61 and L/XL n = 24), the XL alleles were treated as L alleles in this meta-analysis.

5.2.2.5 Other polymorphisms

A number of other polymorphisms have been investigated in the literature, most of which are from candidate genes based on the monoamine, neurotrophic or neuroinflammatory hypotheses of depression (refer to Chapter 2.4). As these have been examined in fewer than three independent studies, meta-analyses were not carried out for these polymorphisms, but their findings are summarised in Table 5.3.

Table 5.3. Summary of findings from included candidate gene studies not in meta-analyses.

Gene	First author (year)	Polymorphism(s)	Phenotype	Association(s)
ACE	López-León (2006)	Ins/Del	DSM-IV MD	N/A
AGTR1	Taylor (2012)	rs2638363, rs10935724, rs1492103, rs718858, rs12721331, rs2675511, rs389566, rs12695902, rs385338	DSM-IV MD	rs10935724: AA genotype in cases>controls (p=0.0487) rs12721331: TT genotype in cases>controls (p=0.0082)
	Taylor (2013)	rs5182, rs5186 (1166A/C)	DSM-IV MD	N/A
AKT1	Pereira (2014)	rs2494731, rs3803304, rs3730358, rs2494738, rs10149779, rs2494746, rs1130214	DSM-IV-TR MD & GDS-15≥6	rs3730358: A allele in cases>controls (p=0.003)
AKTIP	Pereira (2014)	rs9302648, rs7189819	DSM-IV-TR MD & GDS-15≥6	N/A
BDNF	You (2010)	712G/A, 270C/T, 11757G/C	DSM-IV MD & HAM-D≥17	N/A
CNTF	Grünblatt (2006a)	rs1800169 (G/A null mutation)	DSM-IV major/minor depression	N/A
COMT	Pan (2009) Wang (2014)	rs4680 (Val158Met)	NIMH DIS & MADRS DSM-IV MD & HAM-D≥17	N/A
CRP	Almeida (2009b)	rs1130865 (1444C/T), rs1205 (1846G/A)	GDS-15≥7	rs1205: AA genotype in cases>controls (p=0.022)
CRY1	Hua (2014)	rs2287161	DSM-IV-TR MD & HAM-D≥20	CC genotype & C allele in cases>controls (p=0.010 & p=0.012 respectively)
CRY2	Hua (2014)	rs10838524	DSM-IV-TR MD & HAM-D≥20	N/A
IL1B	Hwang (2009)	rs16944 (511C/T)	DSM-IV MD	N/A
IL10	Torres (2013)	rs1800896 (1082G/A)	DSM-IV-TR MD & GDS-15≥6	N/A
LRP1	Schahab (2006)	766C/T	DSM-IV MD	N/A

Gene	First author (year)	Polymorphism(s)	Phenotype	Association(s)
NTRK2	Lin (2009)	rs1187323, rs1187329, rs1545285	DSM-IV MD	rs1187323: CA+CC genotypes & C allele in cases>controls (p=0.0020 & p=0.0042) rs1187329: AA genotype, GA+AA genotypes & A allele in cases>controls (p=0.0160, p=0.0444 & p=0.0089)
PCLO	Hek (2010)	rs2522833	DSM-IV MD or dysthymia	C allele in cases>controls (p=0.0025)
PPARG	Yue (2009)	Pro12Ala	GDS-CD>10	Pro/Ala genotype in cases<controls (p=0.031)
SLC6A4	Jansson (2003)	Exon1B 925C/A	CES-D≥16 or antidepressant use or major/minor depression in medical records	N/A
TNF	Cerri (2010)	rs1800629 (308G/A)	DSM-IV MD & GDS-30≥15	GG genotype in cases>controls (p=0.007)
TPH2	Pereira (2011)	rs4448731, rs4565946, rs11179000, rs7955501, rs10506645, rs4760820, rs1487275, rs10879357	DSM-IV MD & GDS-15≥6	rs4565946: CT genotype in cases<controls (p=0.034) rs11179000: AA genotype & A allele in cases>controls (p=0.025 & p=0.005)
	Wang (2015)	rs4290270, rs7305115	DSM-IV MD & HAM-D≥17	N/A
TEF	Hua (2014)	rs738499	DSM-IV-TR MD & HAM-D≥20	TT genotype and T allele in cases>controls (ps<0.001)
TFCP2	Schahab (2006)	G/A	DSM-IV MD	GG genotype in cases>controls (p=0.042)

Abbreviations: CES-D – Centre for Epidemiologic Studies Depression Scale; DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, 4th edition; DSM-IV-TR – Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision; GDS – Geriatric Depression Scale; HAM-D – Hamilton Rating Scale for Depression; MADRS – Montgomery-Åsberg Depression Scale; MD – major depression; NIMH DIS – National Institute of Mental Health Diagnostic Interview Schedule

5.2.2.6 Genome-wide association studies

Hek et al. (2013) is the only GWAS of depressive symptoms (measured with the CES-D) using predominantly ageing cohorts identified in the search. This GWAS included 17 population-based studies (n = 34,549) in the discovery sample and a total of 2,391,896 SNPs were analysed, none of which reached genome-wide significance. Independent top SNPs with $p < 1 \times 10^{-5}$ in the discovery sample were selected for replication in the replication sample of five studies (n = 16,709); one SNP (rs161645 in the 5q21 region) showed an association with depressive symptoms, yet it was no longer significant after correcting for multiple comparisons. When the discovery and replication samples were pooled (n = 51,258) for a meta-analysis, rs40465, a SNP in high linkage disequilibrium with rs161645, reached genome-wide significance.

5.2.2.7 Sensitivity analysis

Omission of Schmand et al.'s (1998) study increased the OR to 1.53 (95% CI 0.89-2.64, p = 0.12) in the *APOE* ε4 vs ε3 comparison, omission of Taylor et al.'s (2007b) study reduced the OR to 1.24 (95% CI 0.99-1.54, p = 0.06) in the *BDNF* Met vs Val comparison, and omission of Grünblatt et al.'s (2006b) study reduced the OR to 1.22 (95% 0.86-1.74, p = 0.26) in the *SLC6A4* 5-HTTLPR S vs L comparison, all of which became statistically non-significant. However, one possible explanation for these results is that they potentially reflect the loss of statistical power from omission of a study rather than non-robustness of the results given the limited number of studies included and small sample sizes.

5.2.2.8 Potential publication bias

All funnel plots except for the *BDNF* Met/Met vs Val/Val appeared to be reasonably symmetrical (Figure 5.3). While funnel plot asymmetry is often seen as evidence for publication bias, it can be seen that asymmetry in the *BDNF* Met/Met vs Val/Val funnel plot is due to exaggerated effect sizes in smaller studies, a phenomenon commonly observed in meta-analyses (Button et al., 2013).

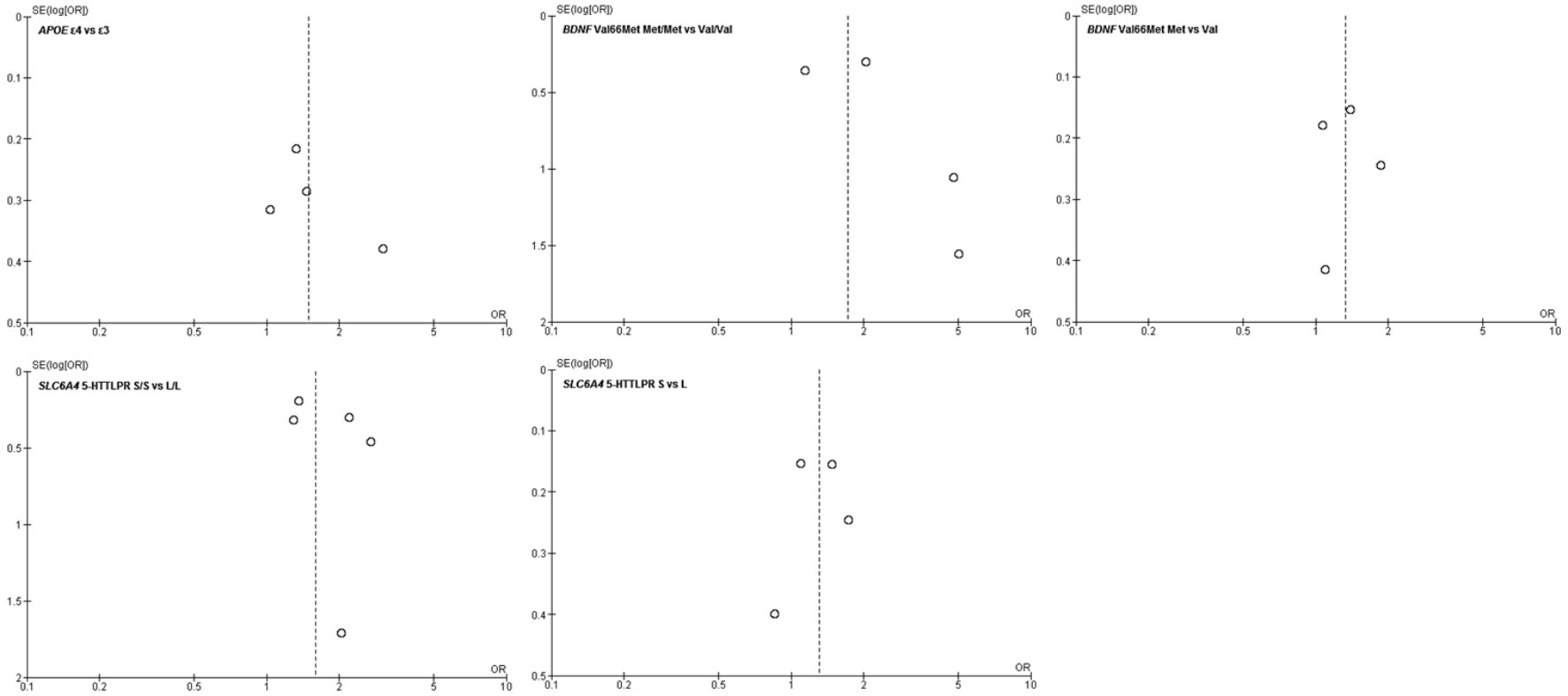

Figure 5.3. Funnel plots of statistically significant meta-analyses.

5.3 Candidate polymorphisms

5.3.1 Methods

5.3.1.1 Participants

Participants were a subsample selected from baseline (Wave 1) of the Sydney MAS. Exclusion criteria were (i) more than 20% missing data on the 15-item Geriatric Depression Scale (GDS-15), (ii) a self-reported diagnosis of schizophrenia or bipolar disorder at Wave 1, (iii) cognitively unclassified at Wave 1 due to too much missing neuropsychological data, missing or invalid instrumental activities of daily living data, or non-English speaking background, (iv) missing genetic data, or (v) of non-Caucasian ancestry. Depression status was defined by a GDS-15 score of six or above or self-reported antidepressant use.

5.3.1.2 Statistical analyses

Candidate polymorphisms identified as significantly associated with LLD in the meta-analysis above (*APOE* ε2/ε3/ε4, *BDNF* Val66Met and rs40465) and additional polymorphisms found to be significantly associated with MDD in an earlier meta-analysis (*GNB3* C825T and *MTHFR* C677T) (López-León et al., 2008) were investigated in relation to LLD. Prior to examining the associations between candidate polymorphisms and LLD, genotype distributions in the control group were assessed for potential deviation from HWE, and those that deviated from HWE were excluded from the analysis. The *SLC6A4* 5-HTTLPR polymorphism was not examined as these data were not available for this sample. Associations between the candidate polymorphisms and LLD were investigated using logistic regression. The dependent variable was dichotomous depression status based on the GDS-15 score or self-reported current antidepressant which was used in all models; the data were coded as 0 = 'not depressed' and 1 = 'depressed'. The predictor variables in each model were the polymorphism of interest, age and sex. The common homozygote was used as the base group in each model. Sensitivity analyses were conducted with depression defined as a GDS-15 score of 12 or above or self-reported antidepressant use. Secondary analyses were also performed using multiple regression with the continuous GDS-15 score as the dependent variable.

5.3.2 Results

5.3.2.1 Sample characteristics

A total of 787 participants were included in this study. There were no significant differences on any of the main demographic variables and the Mini-Mental State Examination (MMSE) (Folstein et al., 1975), a brief screening of cognitive function, between the two groups (Table 5.4).

Table 5.4. Demographic information of the Sydney MAS subsample included in this study (*M* ± *SD* or *n* (%)).

	Control (*n* = 678)	Depressed (*n* = 109)	Test statistic[a]	*p*
Age, years	78.6 ± 4.7	79.0 ± 4.8	35304.00	0.51
Sex, female	383 (56.5)	62 (56.9)	0.006	0.94
Education, years	11.7 ± 3.5	11.0 ± 3.2	33008.50	0.09
Adjusted MMSE total[b]	28.7 ± 1.3	28.5 ± 1.6	35672.00	0.55
GDS-15 total	1.8 ± 1.3	4.5 ± 3.1	17643.50	**<0.001**

[a] Group differences were tested with Mann-Whitney *U* test, except for the Sex variable, which was tested with χ^2.
[b] The MMSE score is adjusted for age, education and non-English speaking background according to Anderson et al. (2007).
Abbreviations: MMSE – Mini-Mental State Examination; GDS-15 – 15-item Geriatric Depression Scale.

5.3.2.2 Genotype frequencies and deviations from Hardy-Weinberg equilibrium

Genotype frequencies for each candidate polymorphism are presented in Table 5.5 below. Genotype distributions of *APOE* ε2/ε3/ε4 (rs429358 and rs7412), *BDNF* Val66Met (rs6265), *GNB3* C825T (rs5443), *MTHFR* C677T (rs1801133) as well as rs40465 were tested for potential deviations from HWE in the control group. None of the SNPs tested deviated from HWE.

Table 5.5. Genotype frequencies for candidate polymorphisms and tests for deviations from Hardy-Weinberg equilibrium.

	Control (*n* = 678)	Depressed (*n* = 109)	
APOE ε2/ε3/ε4 rs429358			
TT	4	0	
CT	108	14	
CC	564	95	$\chi^2 = 0.23$, $p = 0.63$
Missing	2	0	
APOE ε2/ε3/ε4 rs7412			
TT	516	85	
CT	143	22	
CC	15	2	$\chi^2 = 1.80$, $p = 0.18$
Missing	4	0	
BDNF Val66Met			
Val/Val	447	67	
Val/Met	205	38	
Met/Met	26	4	$\chi^2 = 0.17$, $p = 0.68$
GNB3 C825T			
C/C	341	54	
C/T	283	47	
T/T	54	8	$\chi^2 = 0.20$ $p = 0.66$
MTHFR C677T			
C/C	260	36	
C/T	313	54	
T/T	105	19	$\chi^2 = 0.45$, $p = 0.50$
rs40465			
T/T	307	47	
G/T	297	55	
G/G	74	7	$\chi^2 = 0.03$, $p = 0.86$

5.3.2.3 Associations between candidate polymorphisms and late-life depression

Separate logistic regression models were run for *APOE* ε2/ε3/ε4, *BDNF* Val66Met, *GNB3* C825T, *MTHFR* C677T and rs40465, and none of the them were significantly associated with LLD (see Tables 5.6 to 5.11). Both the sensitivity analyses with a more stringent cut-off (Tables B.1 to B.6

in Appendix B) and the secondary analyses with the continuous GDS-15 score as the dependent variable (Tables B.7 to B.12 in Appendix B) showed similar results.

Table 5.6. Logistic regression of *APOE* ε2 carrier status in predicting late-life depression.

Predictor	*B*	*SE*	*p*	OR (95% CI)
APOE ε2+	-0.31	0.31	0.31	0.74 (0.41-1.34)
Age	0.02	0.02	0.41	1.02 (0.98-1.06)
Sex	0.02	0.21	0.93	1.02 (0.68-1.53)
(Constant)	-3.19	1.71	0.06	0.04
Model $\chi^2 = 1.68$, $df = 3$, $p = 0.64$				
-2LL = 631.44, Nagelkerke $R^2 = 0.004$				
Hosmer-Lemeshow test $p = 0.71$				

Table 5.7. Logistic regression of *APOE* ε4 carrier status in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR (95% CI)*
APOE ε4+	-0.07	0.25	0.77	0.93 (0.57-1.52)
Age	0.02	0.02	0.45	1.02 (0.97-1.06)
Sex	0.01	0.21	0.97	1.01 (0.67-1.52)
(Constant)	-3.11	1.71	0.07	0.05
Model $\chi^2 = 0.69$, $df = 3$, $p = 0.88$				
-2LL = 632.43, Nagelkerke $R^2 = 0.002$				
Hosmer-Lemeshow test $p = 0.31$				

Table 5.8. Logistic regression of *BDNF* Val66Met in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR (95% CI)*
BDNF Val66Met				
Val/Met	0.21	0.22	0.34	1.23 (0.80-1.90)
Met/Met	0.003	0.55	1.00	1.00 (0.34-2.97)
Age	0.02	0.02	0.45	1.02 (0.97-1.06)
Sex	0.003	0.21	0.99	1.02 (0.67-1.51)
(Constant)	-3.19	1.71	0.06	0.04
Model $\chi^2 = 1.50$, $df = 4$, $p = 0.83$				
-2LL = 631.61, Nagelkerke $R^2 = 0.003$				
Hosmer-Lemeshow test $p = 0.22$				

Table 5.9. Logistic regression of *GNB3* C825T in predicting late-life depression.

Predictor	B	*SE*	*p*	OR (95% CI)
GNB3 C825T				
C/T	0.05	0.22	0.83	1.05 (0.69-1.60)
T/T	-0.08	0.41	0.84	0.92 (0.42-2.04)
Age	0.02	0.02	0.43	1.02 (0.98-1.06)
Sex	0.01	0.21	0.95	1.01 (0.67-1.52)
(Constant)	-3.19	1.71	0.06	0.04
Model $\chi^2 = 0.72$, $df = 4$, $p = 0.95$				
-2LL = 632.39, Nagelkerke $R^2 = 0.002$				
Hosmer-Lemeshow test $p = 0.13$				

Table 5.10. Logistic regression of *MTHFR* C677T in predicting late-life depression.

Predictor	B	*SE*	*p*	OR (95% CI)
MTHFR C677T				
C/T	0.23	0.23	0.33	1.25 (0.80-1.97)
T/T	0.27	0.31	0.38	1.31 (0.72-2.40)
Age	0.02	0.02	0.44	1.02 (0.98-1.06)
Sex	0.04	0.21	0.87	1.04 (0.69-1.56)
(Constant)	-3.33	1.72	0.05	0.04
Model $\chi^2 = 1.82$, $df = 4$, $p = 0.77$				
-2LL = 631.29, Nagelkerke $R^2 = 0.004$				
Hosmer-Lemeshow test $p = 0.76$				

Table 5.11. Logistic regression of rs40465 in predicting late-life depression.

Predictor	B	*SE*	*p*	OR (95% CI)
rs40465				
T/G	0.19	0.22	0.39	1.20 (0.79-1.83)
G/G	-0.48	0.43	0.26	0.62 (0.27-1.43)
Age	0.02	0.02	0.48	1.02 (0.97-1.06)
Sex	0.02	0.21	0.94	1.02 (0.68-1.53)
(Constant)	-3.08	1.71	0.07	0.05
Model $\chi^2 = 3.58$, $df = 4$, $p = 0.47$				
-2LL = 629.53, Nagelkerke $R^2 = 0.008$				
Hosmer-Lemeshow test: $p = 0.64$				

5.4 Discussion

In this chapter, the existing literature on genetic associations with LLD was reviewed, and meta-analyses were conducted for four risk polymorphisms (*APOE* ε2/ε3/ε4, *BDNF* Val66Met, *MTHFR* C677T, *SLC6A4* 5-HTTLPR). Three of these associations (*APOE* ε2/ε3/ε4, *BDNF* Val66Met and *SLC6A4* 5-HTTLPR) were statistically significant, but the pooled effect sizes were mostly modest.

The identified susceptibility genes in the meta-analysis have been studied extensively in relation to the pathogenesis of neuropsychiatric and neurodegenerative disorders. Apolipoprotein E (APOE) for instance plays important roles in lipoprotein metabolism as well as in the regulation of synaptic plasticity and repair (Bu, 2009), and the *APOE* gene (chromosome 19) is a known susceptibility gene for both sporadic and familial Alzheimer's disease. The *APOE* ε2/ε3/ε4 genotypes are based on a haplotype of two single-nucleotide polymorphisms (rs429358 and rs7412). Early studies examining the relationship between *APOE* genotype and depression have reported positive associations of the ε4 allele with both late-onset MDD (Krishnan et al., 1996) and late-life psychotic depression (Zubenko et al., 1996), but later attempts to replicate these findings showed mixed results. Interestingly, while a previous meta-analysis of genetic association studies of MDD showed a protective effective of the *APOE* ε2 allele (López-León et al., 2008), no evidence of this was found in the meta-analysis here. In contrast, the *APOE* ε4 allele was found to be associated with an increased risk of LLD (pooled OR = 1.49, 95% CI 1.03-2.71). Closer inspection of the characteristics of the studies included in that previous meta-analysis showed that one of the studies involved a younger sample (age = 44.6 ± 16.0 years) (Fan et al., 2006), which showed a very strong protective effect of the ε2 allele (*OR* = 0.03-0.06 depending on whether the analysis was based on genotypes or alleles) and was the main cause for the significant between-study heterogeneity. A reanalysis of such data showed that omission of the study with the younger sample would render the between-study heterogeneity as well as the overall pooled effect size non-significant. However, the significant meta-analysis result here should also be interpreted with caution as *APOE* ε4 is a well-recognised risk factor for Alzheimer's disease (Strittmatter & Roses, 1996) and depressive symptoms are commonly reported in individuals with Alzheimer's disease (including in the preclinical stage) (Berger et

al., 1999; Leyhe et al., 2017). Only one included study has examined this potential confounding relationship, which found the association between the ε4 allele and depression did not survive stratification based on the presence of Alzheimer's disease (Slifer et al., 2009). While studies with individuals with dementia were excluded from this meta-analysis, it cannot be ruled out that some of them may be in the preclinical stage of Alzheimer's disease when assessed.

The brain derived neurotrophic factor (BDNF) is a neurotrophin that is highly expressed in the hippocampus; it plays a pivotal role in the regulation of synaptic plasticity, neurogenesis, neuronal survival and differentiation, and may be important in the pathophysiology of a range of neuropsychiatric disorders including depression (Duman et al., 2016). A common single-nucleotide polymorphism in the *BDNF* gene (chromosome 11) results in the substitution of valine by methionine (Val66Met, rs6265), and the Met allele is associated with decreased activity-dependent secretion of BDNF (Egan et al., 2003), poorer memory performance (Hariri et al., 2003) as well as increased risk for several psychiatric disorders (Gratacòs et al., 2007). Two earlier meta-analyses of genetic associations of MDD showed no significant association between the *BDNF* Val66Met polymorphism and MDD in younger samples, but a more recent meta-analysis focusing on LLD found the Met allele was associated with an increased risk for LLD (Pei et al., 2012). The findings in this chapter are concordant with the results of the latter meta-analysis despite the different studies included.

The 5-HTTLPR polymorphism is a repeat length polymorphism located in the promoter region of the *SLC6A4* gene (chromosome 17), and the two common alleles correspond to either a 44-base pair insertion (Long allele; 16-repeat) or deletion (Short allele; 14-repeat). The Short allele is associated with lower transcription of the serotonin transporter, resulting in reduced serotonin uptake and expression (Heils et al., 1996; Lesch et al., 1996). While studies investigating the 5-HTTLPR polymorphism in depression commonly use a biallelic grouping, comparing the disease risk between S allele and L allele carriers, there have been reports of several variants of the 14- and 16-repeat, as well as other types of alleles (15-, 18-, 19-, 20- and 22-repeat), although they are rarely observed in Caucasians (Delbruck et al., 1997; Kunugi et al., 1997; Michaelovsky et al., 1999; Nakamura et al., 2000). Moreover, additional functional polymorphisms at rs25531,

rs2020933 and rs8073965 have been reported to also contribute to the transcriptional variation of *SCL6A4* (Martin et al., 2007; Wendland et al., 2006). Another study established that the *SCL6A4* 5-HTTLPR polymorphism is functionally triallelic, with the three alleles being L_A, L_G, and S, where the L_G allele is assumed to be low-expressing like the S allele (Hu et al., 2005). This was caused by an A>G substitution at position 6 within the first of two 22-base pair repeats in the L allele (Nakamura et al., 2000), which resulted in a functional AP2 transcription factor binding site. While studies that failed to take the triallelic nature of this polymorphism into account risk skewing the results artificially due to the functional misclassification of the L_G allele carriers, depression studies that used the triallelic grouping interestingly tend to report smaller effect sizes than those that used the biallelic grouping (Clarke et al., 2010).

The only GWAS involving predominantly older cohorts found preliminary evidence for an association between the 5q21 region and late-life depressive symptoms (Hek et al., 2013). The 5q21 region identified is located in a gene desert and no association between its closest gene *NUDT12* and any psychiatric disorder has previously been reported. It is perhaps unsurprising that none of the significant candidate polymorphisms reached genome-wide significance in the single GWAS identified in the review. A prior study that tried to use data from a GWAS as a large-scale replication of significant findings reported in candidate genes studies of MDD similarly found only minimal support for few of the candidate genes in the literature (Bosker et al., 2011). The authors argue that the most likely explanation for this is publication bias. However, a number of other explanations are also highly plausible. Firstly, depression is a heterogeneous disorder, and how the phenotype is defined can have an effect on the magnitude of the association. All the studies included in the GWAS identified assessed depressive symptoms using the Centre for Epidemiological Studies Depression Scale (CES-D), whereas the meta-analyses in this chapter included studies of depression based on the Diagnostic and Statistical Manual of Mental Disorders (DSM) diagnosis as well as depressive symptoms based on self-rated instruments. Furthermore, insufficient statistical power is a common issue in both candidate polymorphism studies and GWAS. Power calculations suggest a sample of 3600 cases and 3600 controls are required to achieve 90% power to detect a genotypic relative risk of 1.16 at minor allele frequency of 0.30 in a candidate gene study (Major Depressive Disorder Working Group of

the Psychiatric GWAS Consortium, 2013). MDD being a highly prevalent and modestly to moderately heritable disorder, it has been estimated the sample size required for a GWAS of MDD is 2.4- to 5-fold greater compared with schizophrenia, which is likely to be achieved only through the use of national registries or electronic health records (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013).

Overall, the results of the systematic review and meta-analysis here show that the genetics of LLD is insufficiently investigated, with limited replication studies reported. It is therefore unsurprising that no robust associations have been identified to date. While meta-analysis is a potentially powerful statistical tool, it is limited by the quality of studies that are included in the first place. In this chapter, each of the meta-analyses only included a few studies with rather small sample sizes; as a consequence the pooled sample sizes may still be too small for reliable detection of modest to moderate genetic effects (Zintzaras & Lau, 2007). The pooled ORs here also provide some indication that the effect sizes may be inflated due to the small sample sizes and could potentially represent false positive (type I errors); under the common disease/common variant hypothesis, the contribution of each risk variant is expected to be rather small, and prior studies on genetic associations between common variants and common diseases generally report ORs in the range of 1.1-1.4 or less commonly 1.4-2.0 (Ioannidis, 2008). The risk polymorphisms identified in this review also represent just a very small percentage of the variance in liability to disease, as it is believed that thousands of independent loci contribute to depression susceptibility, most of them being common variants of small effect (Flint & Kendler, 2014). This has been demonstrated in a study using the genetic risk profiling approach, which found that a cluster of selected top SNPs explained only 0.7%-1.0% of the variance in depression in two samples of older adults (Demirkan et al., 2011).

In the second half to this chapter, polymorphisms identified as significantly associated with LLD in the review (*APOE* ε2/ε3/ε4, *BDNF* Val66Met and rs40465 on Chromosome 5) and those associated with MDD in an earlier meta-analysis (*GNB3* C825T and *MTHFR* C677T) (López-León et al., 2008) were investigated in a subsample of the Sydney MAS. None of these polymorphisms significantly predicted LLD. As discussed above, publication bias and

insufficient statistical power are likely to be the reasons behind inconsistent results reported in candidate polymorphism studies.

A clear limitation of all the studies included in this review as well as the candidate polymorphism studies carried out in this chapter is inadequate statistical power, therefore we should exercise caution in the interpretation of these findings. It would be premature to consider the significant associations definitive in the aetiology of LLD as effect sizes are possibly inflated, whereas a non-significant association in the meta-analysis or the candidate polymorphism study also does not necessarily indicate that a specific polymorphism does not play a role in the pathogenesis of LLD. Before we are able to perform meta-analysis on data from multiple adequately powered studies, these associations cannot be convincingly confirmed or refuted. Larger and more replication studies are required in the future to provide stronger evidence of genetic associations with LLD, which would also allow for meaningful stratified analyses based on depression subtype (such as early-onset recurrent versus late-onset depression), ethnicity or sex to limit heterogeneity in the sample.

6 IMPACT OF EARLY-LIFE TRAUMA ON LATE-LIFE DEPRESSION

6.1 Introduction

Examination of the genetic and environmental influences on late-life depression (LLD) in Chapter 4 revealed that environmental factors explain a substantial proportion of the variation in LLD; less than perfect concordance in MZ twins also suggest that environmental factors play an important role in the aetiology of LLD. Many environmental risk factors for mental disorders including depression have been identified to date such as low birth weight, birth complications, and exposure to family conflict. Adversity that occurs in early life is considered to be particularly relevant, as it is hypothesised that such events occurring at critical stages of brain development may lead to lasting structural and functional changes in the brain, for instance HPA axis dysregulation or changes to immune function, altering biological processes in relation to stressful events. This chapter will investigate the effects of a specific type of environmental risk factor – early-life trauma, as well as potential gene-environment interactions on LLD.

6.1.1 Childhood maltreatment

Research suggests exposure to childhood maltreatment is linked to psychopathology later in adulthood (Kendler et al., 2000; Kessler et al., 1997; Reeve & van Gool, 2013). Meta-analyses have shown that individuals with a history of childhood maltreatment are more likely to develop depression in adulthood, and more likely to have a severe course of depression with chronicity, atypical features (significant weight gain or increased appetite, hypersomnia, leaden paralysis, or interpersonal rejection sensitivity), and poor treatment response (Nanni et al., 2012; Nelson et al., 2017; Withers et al., 2013). However, most of the research has focused on examining the impact of such experiences on depression in adolescence and young adulthood, and the role of childhood maltreatment in the development of LLD has been insufficiently investigated.

Among the limited studies that have been conducted, there is some evidence that various types of childhood maltreatment are associated with LLD or depressive symptoms. A prospective ageing cohort study from the Netherlands showed that emotional neglect, psychological abuse, physical abuse, and sexual abuse all predicted depression (odds ratios (*OR*s) ranged from 5.35

(95% confidence interval (CI) 2.36-12.14) for sexual abuse to 13.71 (95% CI 3.25-57.91) for physical abuse). The association remained significant when the analysis was stratified by age of onset. Within the depressed group, all types of abuse were related to significantly more severe depression, a younger age of onset, a higher number of depressive episodes, a lower sense of mastery, as well as a higher number of chronic diseases (Comijs et al., 2013). A population-based study examining LLD found that repeated exposure to six types of adverse childhood experiences (namely physical abuse between parents, physical abuse by a parent, being sworn at by a parent, being touched sexually by an adult, being forced to sexually touch an adult, and being forced into sexual intercourse) were individually associated with a greater risk of developing depression (adjusted ORs ranged from 2.41 to 9.78, all $p < 0.001$). When all types of abuse were entered into the same model, repeated physical abuse by a parent and repeated forced sexual intercourse remained as significant predictors of LLD (Ege et al., 2015).

This association between childhood maltreatment and LLD may be mediated by a range of factors. For instance, the association between a history of childhood sexual abuse and depressive symptom severity was partially accounted for by neuroticism in a sample of older psychiatric patients, although the association remained significant after taking neuroticism into account (Gamble et al., 2006). Another study that looked at the relationship between a childhood abuse composite and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) depression diagnosis (major depression, minor depression or dysthymia) found the relationship became non-significant once mediating variables comprising depression severity, age of depression onset, neuroticism, loneliness, social network size and the number of chronic diseases were included in the model (Wielaard et al., 2017).

6.1.2 War-related trauma

Aside from interpersonal trauma, impersonal trauma such as exposure to war-related trauma also has a huge impact on the health of older adults. In particular, the "concentration camp syndrome" (also known as *Konzentrationslagersyndrom* or KZ-syndrome) in Holocaust survivors has been well documented in the literature, including symptoms such as dysphoric mood, emotional lability, sleep disturbances, and cognitive deficits (Chodoff, 1963; Eitinger,

1961), which is now classified as post-traumatic stress disorder in the current psychiatric nosology. In the past decades, there has been growing recognition that the consequences of these traumatic experiences persist well into middle age and later life, with epidemiological studies around the world reporting that between 40% and 86% of survivors still display concentration camp symptoms 30 to 50 years after leaving the concentration camps (Bower, 1994; Jabłoński et al., 2016; Kuch & Cox, 1992; Robinson et al., 1994; Robinson et al., 1990). A comprehensive meta-analysis found significant negative long-term effects of the Holocaust on survivors' health and well-being, including poorer psychological well-being (Cohen's $d = 0.14$, 95% CI 0.03-0.24, $p < 0.05$), more post-traumatic stress symptoms (Cohen's $d = 0.72$, 95% CI 0.46-0.98, $p < 0.01$), as well as greater psychopathological symptomatology (Cohen's $d = 0.33$, 95% CI 0.23-0.44, $p < 0.01$) (Barel et al., 2010). Similarly, in studies that specifically used depression or depressive symptoms as an outcome variable, Holocaust survivors or those who have had extreme experiences during World War II were shown to be at higher risk of depression or had higher levels of depressive symptoms (Amir & Lev-Wiesel, 2003; Beekman et al., 1995). However, few studies have explored the influence of war-related trauma on LLD.

6.1.3 Gene-environment interactions in depression

The literature has shown that early-life adversity is a robust predictor of adolescent and adult depression (Heim & Binder, 2012), and the majority of studies examining gene-environment interactions involving early-life adversity or stressful life events have focused on the solute carrier family 6 member 4 (*SLC6A4*) 5-HTTLPR polymorphism. The 5-HTTLPR polymorphism is a repeat length polymorphism located in the promotor region of the *SLC6A4* gene, and is associated with transcription activity of the serotonin transporter. The short (S) allele is associated with reduced serotonin uptake and expression. Few studies have explored gene-environment interactions in LLD. All of those examining the genetic moderation of the association between early-life adversity or stressful life events and LLD involved the 5-HTTLPR polymorphism, and the results are mixed.

Kim et al. (2007) was the first study that attempted to replicate the 5-HTTLPR x stressful life events finding in LLD first reported in Caspi et al. (2003) in depression early in life. In addition,

it tested potential modifying effects of the brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism, since *BDNF* is known to play a role in the regulation of synaptic plasticity, neurogenesis, neuronal survival and differentiation. While it reported significant *SLC6A4* x stressful life events, *BDNF* x stressful life events as well as a 3-way *SLC6A4* x *BDNF* x stressful life events effects, the results need to be interpreted with caution as contrary to what was reported in the paper, the sample showed significant deviations from Hardy-Weinberg equilibrium (HWE) for the *BDNF* genotype. A larger French community study that also tried to replicate the *SLC6A4* 5-HTTLPR x life events finding using recent life events that occurred within 12 months found no significant interaction (Wendland et al., 2006). As the G variant of the rs25531 SNP has previously been reported to reduce *SLC6A4* messenger ribonucleic acid (mRNA) transcription for the 5-HTTLPR long allele and is assumed to be similarly low-expressing like the S allele (Hu et al., 2005), the study also examined the gene-environment interaction with the *SLC6A4* genotype reclassified based on the rs25531 functional polymorphism, and no significant interaction was observed (Wendland et al., 2006). Another French study examined reported significant 5-HTTLPR x childhood traumatic experiences interactions for "parents too often shared their problems with children" and "poverty and financial difficulties", but a significantly increased risk of depression was only observed in those with the S/L genotype, with a non-significant trend for the interaction also observed in the L/L group. This is contrary to findings of earlier gene-environment studies that reported greater susceptibility for depression in carriers of the S allele (Ritchie et al., 2009).

On the other hand, only one study has investigated gene-environment interactions involving war exposure in LLD. In a French community-based study of older adults, Artero et al. (2011) explored the *SLC6A4* 5-HTTLPR x war exposure interaction in LLD. War exposure was defined as repatriation from Algeria to France and/or experience of at least one war-related traumatic event. The *SLC6A4* x war exposure interaction was highly significant, with significant associations between war exposure and lifetime depression observed both in the S/S and S/L groups (*OR* = 2.34, 95% CI 1.24-4.32 and OR = 1.64, 95% CI 1.16-2.31 respectively).

As reviewed above, previous gene-environment interaction studies of candidate polymorphisms with stressful life events in LLD have focused on the 5-HTTLPR polymorphism. Aside from the Kim et al. (2007) study which has certain methodological issues, no study to date has examined gene-environment interactions of other candidate polymorphisms of LLD with early-life adversity. The aims of this chapter are (i) to estimate the prevalence of childhood maltreatment in a cohort of older Australians, (ii) to replicate previously reported associations between different types of early-life adversity, including subtypes of childhood maltreatment and exposure to Holocaust trauma, and LLD, and (iii) to explore whether early-life adversity interacts with the candidate polymorphisms of LLD examined in Chapter 5 to contribute to increased vulnerability for LLD.

6.2 Methods

6.2.1 Participants

Participants are a subsample selected from Wave 4 of Sydney MAS. Exclusion criteria were (i) more than 20% missing data on the 15-item Geriatric Depression Scale (GDS-15), (ii) missing data on any of the Childhood Trauma Questionnaire-Short Form (CTQ-SF) subscales, (iii) missing data on the Holocaust trauma item, (iv) a self-reported diagnosis of schizophrenia or bipolar disorder at Wave 1, (v) a consensus cognitive diagnosis of dementia at Wave 4, (vi) genetic data unavailable, or (vii) of non-Caucasian ancestry. A total of 458 participants were included in this study. Sample demographics are shown in Table 6.1 below. The depressed group had a higher percentage of females, a higher GDS-15 score and a higher percentage of Holocaust survivors.

Table 6.1. Sample demographics (*M* ± *SD* or *n* (%)).

	Non-depressed (*n* = 363)	Depressed (*n* = 95)	Test statistic[a]	*p*
Age, years	83.5 ± 4.4	83.9 ± 4.5	16487.0	0.51
Sex, female	200 (55.1)	63 (66.3)	3.9	**<0.05**
Education, years	12.0 ± 3.5	11.6 ± 3.2	16408.5	0.47
Adjusted MMSE total	29.1 ± 1.2	29.1 ± 1.1	15972.5	0.76
GDS-15 total	2.1 ± 1.3	5.0 ± 3.3	7857.0	**<0.01**
CTQ total	42.1 ± 6.3	44.4 ± 9.1	15088.0	0.06
Holocaust survivor	27 (7.4)	14 (14.7)	4.9	**0.03**

[a] Mann-Whitney *U* test for continuous variables and Pearson's χ^2 for dichotomous variables.
Note: Significant results are indicated in bold.

6.2.2 Measures

Depression was defined as a GDS-15 total score of six or above or self-reported current antidepressant use. History of childhood maltreatment was assessed using the CTQ-SF, and cut-off scores reported in Walker et al. (1999) were used to create dichotomous variables for each subscale. The psychometric properties of these measures have already been reported in Chapter 3. Experience of Holocaust trauma was based on one item in a self-reported early life events questionnaire that assessed various types of stressful early life events including birth problems, family dysfunction, disaster, and war events. The actual wording used in that item was "Were you a Holocaust victim?" with the definition of a Holocaust victim being "a person who lived in a country at the time when it was under Nazi regime, under Nazi occupation, or under the regime of Nazi collaborators or who fled a country or region not under Nazi rule or occupation due to Nazi rule or Nazi occupation." Responses were coded as 0 = 'No', 1 = 'Yes' and 888 = 'Don't know'.

Candidate polymorphisms included in this analysis were single-nucleotide polymorphisms (SNPs) identified in the meta-analysis presented in Chapter 5 as well as an earlier meta-analysis of genetic association studies of major depressive disorder (MDD) (López-León et al., 2008).

6.2.3 Testing for deviations from Hardy-Weinberg Equilibrium

For data quality control, genotype distributions of *APOE* ε2/ε3/ε4 (rs429358 and rs7412), *BDNF* Val66Met (rs6265), *GNB3* C825T (rs5443), *MTHFR* C677T (rs1801133) as well as rs40465 (located in a gene desert in the 5q21 region) were tested for potential deviations from HWE in the controls using χ^2. The results are presented in Table 6.2 below. None of the candidate polymorphisms tested deviated from HWE.

Table 6.2. Genotype distributions and tests for deviation from Hardy-Weinberg equilibrium for *APOE* ε2/ε3/ε4 (rs429358 and rs7412), *BDNF* Val66Met, *GNB3* C825T, *MTHFR* C677T and rs40465.

Polymorphism	Common homozygotes	Heterozygotes	Rare homozygotes	χ^2 (1df)	*p*
APOE ε2/ε3/ε4					
- rs429358	0	52	311	2.16	0.14
- rs7412	295	63	5	0.60	0.44
BDNF Val66Met	237	116	10	0.89	0.35
GNB3 C825T	160	174	29	3.79	0.05
MTHFR C677T	139	164	60	0.96	0.33
rs40465	164	162	37	0.11	0.75

6.2.4 Statistical analyses

Prevalence of the five subtypes of childhood maltreatment assessed by the CTQ-SF and self-identification as a Holocaust victim was examined. Associations between the candidate polymorphisms and LLD were investigated using logistic regressions. The dependent variable was the dichotomous depression variable based on GDS-15 score and self-reported current antidepressant use in all models; the data was coded as 0 = 'not depressed' and 1 = 'depressed'. The first model was tested with all dichotomous CTQ subscale variables as independent variables, and age and sex entered as covariates. A second model was tested with Holocaust trauma as the independent variable, again with age and sex as covariates. Effect sizes were quantified using ORs with one-unit increase in the predictor variable. As a continuous variable was included in the models (i.e. age), model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test ($p < 0.05$ indicates a poor fit).

For the gene-environment interaction analysis, a dichotomous composite childhood maltreatment variable based on the five CTQ-SF subscales was created and coded as 0 = 'no history of maltreatment' and 1 = 'history of maltreatment'. Two models were tested for each polymorphism, with the first model testing for a genotype x childhood maltreatment interaction, and the second model testing for a genotype x Holocaust trauma interaction.

Secondary analyses were performed using the continuous GDS-15 score as the dependent variable for all associations tested.

6.3 Results

6.3.1 Prevalence of childhood maltreatment

The prevalence of positive history of childhood maltreatment based on the cut-offs of Walker et al. (1999) is reported in Table 6.3 below.

Table 6.3. Prevalence of childhood maltreatment assessed using the CTQ-SF (*n* (%)).

	Non-depressed (*n* = 363)	Depressed (*n* = 95)	Total (*n* = 458)
Emotional abuse	20 (5.5)	16 (16.8)	36 (7.9)
Physical abuse	29 (8.0)	6 (6.3)	35 (7.6)
Sexual abuse	15 (4.1)	7 (7.4)	22 (4.8)
Emotional neglect	33 (9.1)	10 (10.5)	43 (9.4)
Physical neglect	26 (7.2)	10 (10.5)	36 (7.9)

6.3.2 Effects of childhood maltreatment and Holocaust trauma on late-life depression

The main effects of childhood maltreatment and Holocaust trauma on LLD were examined in two separate binary logistic regression models, with age and sex entered as covariates. Out of the five types of childhood maltreatment assessed in the CTQ-SF, only emotional abuse significantly predicted LLD in the Sydney MAS cohort (B = 1.40, Wald = 11.22, $p < 0.01$) (see Table 6.4). Exposure to Holocaust trauma also significantly predicted LLD (B = 0.81, Wald = 5.12, $p = 0.02$) (see Table 6.5). Significant results are indicated in bold.

Table 6.4. Logistic regression of childhood maltreatment in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
Emotional abuse	1.40	0.42	**<0.01**	**4.05 (1.79-9.16)**
Physical abuse	-0.90	0.57	0.11	0.41 (0.13-1.24)
Sexual abuse	0.54	0.54	0.32	1.72 (0.59-5.00)
Emotional neglect	-0.29	0.48	0.54	0.75 (0.30-1.90)
Physical neglect	0.39	0.48	0.41	1.48 (0.58-3.76)
Age	0.02	0.03	0.48	1.02 (0.97-1.07)
Sex	0.43	0.25	0.08	1.54 (0.95-2.51)
(Constant)	-3.31	2.25	0.14	0.04
Model $\chi^2 = 19.44$, $df = 7$, $p = 0.007$				
-2LL = 448.20, Nagelkerke $R^2 = 0.07$				
Hosmer-Lemeshow test $p = 0.49$				

Table 6.5. Logistic regression of Holocaust trauma in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
Holocaust trauma	0.81	0.36	**0.02**	**2.24 (1.11-4.51)**
Age	0.02	0.03	0.55	1.02 (0.97-1.07)
Sex	0.51	0.24	**0.04**	**1.67 (1.03-2.69)**
(Constant)	-3.04	2.20	0.17	0.05
Model $\chi^2 = 9.27$, $df = 3$, $p = 0.03$				
-2LL = 458.37, Nagelkerke $R^2 = 0.03$				
Hosmer-Lemeshow test $p = 0.78$				

Secondary analyses on the effects of early-life trauma showed similar results, but additionally showed that sexual abuse and age significantly predicted GDS-15 scores. Sex, however, was not a significant predictor (see Appendix B).

6.3.3 Gene-environment interactions

Potential gene-environment interactions between the candidate polymorphism of interest and childhood maltreatment or Holocaust trauma were tested in separate binary logistic regression models. The results are presented in Tables 6.6 to 6.17, which show the candidate polymorphism main effect, the early-life trauma main effect, the gene-environment interaction effect, independent of the effects of other predictors. Of the 12 models tested, only four were

statistically significant (Tables 6.9, 6.11, 6.14 and 6.15), and none of the gene-environment interactions significantly predicted LLD. Secondary analyses for gene-environment interactions showed similar results, with all interaction effects tested except for the *BDNF* Val66Met x childhood maltreatment interaction being non-significant. However, given the small sample size in this study, it is possible that this finding is a spurious association.

Table 6.6. Logistic regression of the interaction between *APOE* ε2 allele and childhood maltreatment in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
ε2+	0.17	0.36	0.64	1.18 (0.58-2.39)
Maltreatment	0.46	0.28	0.10	1.58 (0.91-2.75)
ε2+ x maltreatment	-0.75	0.90	0.41	0.48 (0.08-2.76)
Age	0.02	0.03	0.41	1.02 (0.97-1.08)
Sex	0.49	0.24	**0.04**	**1.63 (1.01-2.63)**
(Constant)	-3.55	2.19	0.10	0.03
Model $\chi^2 = 7.19$, $df = 5$, $p = 0.09$				
-2LL = 460.45, Nagelkerke $R^2 = 0.02$				
Hosmer-Lemeshow test $p = 0.52$				

Table 6.7. Logistic regression of the interaction between *APOE* ε2 allele and Holocaust trauma in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
ε2+	-0.05	0.36	0.90	0.96 (0.47-1.95)
Holocaust trauma	0.81	0.40	**0.04**	**2.25 (1.03-4.95)**
ε2+ x Holocaust trauma	-0.01	0.89	0.99	0.99 (0.18-5.64)
Age	0.02	0.03	0.56	1.02 (0.97-1.07)
Sex	0.51	0.24	**0.04**	**1.67 (1.03-2.70)**
(Constant)	-3.02	2.21	0.05	
Model $\chi^2 = 9.29$, $df = 5$, $p = 0.10$				
-2LL = 458.35, Nagelkerke $R^2 = 0.03$				
Hosmer-Lemeshow test $p = 0.80$				

Table 6.8. Logistic regression of the interaction between *APOE* ε4 allele and childhood maltreatment in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
ε4+	0.67	0.31	**0.03**	**1.95 (1.07-3.57)**
Maltreatment	0.54	0.30	0.08	1.71 (0.95-3.08)
ε4+ x maltreatment	-0.65	0.66	0.32	0.52 (0.14-1.90)
Age	0.03	0.03	0.29	1.03 (0.98-1.08)
Sex	0.46	0.24	0.06	1.58 (0.98-2.54)
(Constant)	-4.21	2.22	0.06	0.02
Model $\chi^2 = 10.96$, $df = 5$, $p = 0.05$				
-2LL = 456.68, Nagelkerke $R^2 = 0.04$				
Hosmer-Lemeshow test $p = 0.46$				

Table 6.9. Logistic regression of the interaction between *APOE* ε4 allele and Holocaust trauma in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
ε4+	0.54	0.29	0.06	1.71 (0.98-2.99)
Holocaust trauma	0.85	0.40	**0.03**	**2.35 (1.07-5.15)**
ε4+ x Holocaust trauma	-0.13	0.91	0.88	0.88 (0.15-5.15)
Age	0.02	0.03	0.43	1.02 (0.97-1.08)
Sex	0.50	0.25	**0.04**	**1.65 (1.02-2.67)**
(Constant)	-3.60	2.24	0.11	0.03
Model $\chi^2 = 12.85$, $df = 5$, $p = 0.03$				
-2LL = 454.79, Nagelkerke $R^2 = 0.04$				
Hosmer-Lemeshow test $p = 0.61$				

Table 6.10. Logistic regression of the interaction between *BDNF* Val66Met genotype and childhood maltreatment in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
BDNF				
Val/Met	-0.32	0.31	0.30	0.73 (0.40-1.33)
Met/Met	0.54	0.72	0.45	1.72 (0.42-7.00)
Maltreatment	0.31	0.33	0.35	1.36 (0.72-2.58)
BDNF x maltreatment				
Val/Met x maltreatment	0.15	0.60	0.81	1.16 (0.36-3.79)
Met/Met x maltreatment	0.22	1.20	0.86	1.24 (0.12-12.95)
Age	0.02	0.03	0.38	1.02 (0.97-1.08)
Sex	0.51	0.24	**0.04**	**1.66 (1.03-2.68)**
(Constant)	-3.56	2.20	0.11	0.03
Model $\chi^2 = 9.07$, $df = 7$, $p = 0.25$				
-2LL = 458.57, Nagelkerke $R^2 = 0.03$				
Hosmer-Lemeshow test $p = 0.50$				

Table 6.11. Logistic regression of the interaction between *BDNF* Val66Met genotype and Holocaust trauma in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
BDNF				
Val/Met	-0.17	0.27	0.54	0.85 (0.49-1.45)
Met/Met	0.25	0.68	0.72	1.28 (0.34-4.86)
Holocaust trauma	0.79	0.41	0.05	2.21 (0.99-4.90)
BDNF x Holocaust victim				
Val/Met x Holocaust trauma	-1.08	1.18	0.36	0.34 (0.03-3.44)
Met/Met x Holocaust trauma	21.59	28284.60	1.00	0.00
Age	0.02	0.03	0.52	1.02 (0.97-1.07)
Sex	0.54	0.25	**0.03**	**1.71 (1.05-2.78)**
(Constant)	-3.14	2.22	0.16	0.04
Model $\chi^2 = 15.75$, $df = 7$, $p = 0.03$				
-2LL = 451.889, Nagelkerke $R^2 = 0.05$				
Hosmer-Lemeshow test $p = 0.32$				

Table 6.12. Logistic regression of the interaction between *GNB3* C825T genotype and childhood maltreatment in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
GNB3				
C/T	0.20	0.28	0.49	1.22 (0.70-2.12)
T/T	0.001	0.54	1.00	1.00 (0.35-2.89)
Maltreatment	0.67	0.38	0.08	1.96 (0.92-4.17)
GNB3 x maltreatment				
C/T x maltreatment	-0.49	0.55	0.38	0.61 (0.21-1.81)
T/T x maltreatment	-1.12	1.25	0.37	0.33 (0.03-3.76)
Age	0.03	0.03	0.34	1.03 (0.97-1.08)
Sex	0.49	0.24	**0.04**	**1.63 (1.01-2.63)**
(Constant)	-3.90	2.22	0.08	0.02
Model $\chi^2 = 8.30$, $df = 7$, $p = 0.31$				
-2LL = 459.34, Nagelkerke $R^2 = 0.03$				
Hosmer-Lemeshow test $p = 0.71$				

Table 6.13. Logistic regression of the interaction between *GNB3* C825T genotype and Holocaust trauma in predicting late life-depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
GNB3				
C/T	-0.04	0.26	0.87	0.96 (0.58-1.59)
T/T	-0.09	0.49	0.86	0.92 (0.35-2.41)
Holocaust trauma	0.72	0.65	0.26	2.06 (0.58-7.32)
GNB3 x Holocaust trauma				
C/T x Holocaust trauma	0.31	0.78	0.69	1.37 (0.29-6.34)
T/T x Holocaust trauma	-20.45	23105.28	1.00	0.00
Age	0.01	0.03	0.60	1.01 (0.96-1.07)
Sex	0.53	0.25	**0.03**	**1.69 (1.05-2.73)**
(Constant)	-2.88	2.23	0.20	0.06
Model $\chi^2 = 12.20$, $df = 7$, $p = 0.09$				
-2LL = 455.44, Nagelkerke $R^2 = 0.04$				
Hosmer-Lemeshow test $p = 0.73$				

Table 6.14. Logistic regression of the interaction between *MTHFR* C677T genotype and childhood maltreatment in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
MTHFR				
C/T	0.33	0.33	0.31	1.39 (0.74-2.64)
T/T	0.90	0.37	**0.02**	**2.45 (1.18-5.07)**
Maltreatment	0.44	0.46	0.34	1.55 (0.64-3.79)
MTHFR x maltreatment				
C/T x maltreatment	-0.08	0.61	0.90	0.93 (0.28-3.06)
T/T x maltreatment	0.26	0.76	0.73	1.30 (0.29-5.75)
Age	0.02	0.03	0.46	1.02 (0.97-1.07)
Sex	0.57	0.25	**0.02**	**1.77 (1.09-2.88)**
(Constant)	-3.75	2.22	0.09	0.02
Model $\chi^2 = 15.18$, $df = 7$, $p = 0.03$				
-2LL = 452.46, Nagelkerke $R^2 = 0.05$				
Hosmer-Lemeshow test $p = 0.26$				

Table 6.15. Logistic regression of the interaction between *MTHFR* C677T genotype and Holocaust trauma in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
MTHFR				
C/T	0.25	0.29	0.38	1.29 (0.73-2.27)
T/T	0.85	0.35	**0.01**	**2.34 (1.19-4.63)**
Holocaust trauma	0.83	0.87	0.34	2.29 (0.41-12.70)
MTHFR x Holocaust trauma				
C/T x Holocaust trauma	-0.07	1.03	0.95	0.94 (0.13-7.01)
T/T x Holocaust trauma	-0.39	1.06	0.71	0.68 (0.09-5.39)
Age	0.02	0.03	0.56	1.02 (0.96-1.07)
Sex	0.58	0.25	**0.02**	**1.78 (1.10-2.90)**
(Constant)	-3.37	2.25	0.13	0.03
Model $\chi^2 = 15.42$, $df = 7$, $p = 0.03$				
-2LL = 452.22, Nagelkerke $R^2 = 0.05$				
Hosmer-Lemeshow test $p = 0.61$				

Table 6.16. Logistic regression of the interaction between rs40465 and childhood maltreatment in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
rs40465				
G/T	0.30	0.29	0.29	1.35 (0.77-2.36)
G/G	-0.07	0.50	0.90	0.94 (0.35-2.48)
Maltreatment	0.68	0.38	0.08	1.97 (0.93-4.17)
rs40465 x maltreatment				
G/T x maltreatment	-1.00	0.60	0.10	0.37 (0.11-1.20)
G/G x maltreatment	0.71	0.84	0.40	2.03 (0.39-10.47)
Age	0.02	0.03	0.56	1.02 (0.97-1.07)
Sex	0.50	0.25	**0.04**	**1.66 (1.02-2.67)**
(Constant)	-3.15	2.21	0.15	0.04
Model $\chi^2 = 11.57$, $df = 7$, $p = 0.12$				
-2LL = 456.07, Nagelkerke $R^2 = 0.04$				
Hosmer-Lemeshow test $p = 0.51$				

Table 6.17. Logistic regression of the interaction between rs40465 and Holocaust trauma in predicting late-life depression.

Predictor	*B*	*SE*	*p*	*OR* (95% CI)
rs40465				
G/T	0.05	0.27	0.86	1.05 (0.62-1.76)
G/G	0.25	0.41	0.53	1.29 (0.58-2.86)
Holocaust trauma	1.21	0.62	0.05	3.35 (0.99-11.36)
rs40465 x Holocaust trauma				
G/T x Holocaust trauma	-0.51	0.78	0.52	0.60 (0.13-2.78)
G/G x Holocaust trauma	-1.07	1.36	0.43	0.34 (0.02-4.98)
Age	0.02	0.03	0.55	1.02 (0.97-1.07)
Sex	0.52	0.25	**0.04**	**1.68 (1.04-2.71)**
Constant	-3.10	2.21	0.16	0.05
Model $\chi^2 = 10.22$, $df = 7$, $p = 0.18$				
-2LL = 457.42, Nagelkerke $R^2 = 0.04$				
Hosmer-Lemeshow test $p = 0.06$				

6.4 Discussion

This chapter estimated the prevalence of childhood maltreatment in a community sample of older Australians, and studied the associations between experiences of childhood maltreatment or Holocaust trauma and LLD, as well as potential gene-environment interactions with candidate polymorphisms of LLD. While a history of emotional abuse and exposure to Holocaust trauma both predicted LLD independently, none of the gene-environment interactions tested were statistically significant.

The prevalence rates of the five types of childhood maltreatment observed in this study as assessed using the CTQ-SF are relatively low, ranging from around 5% to 9%. Although no methodologically rigorous nationwide prevalence study of childhood abuse and neglect has been conducted to date in Australia (Child Family Community Australia, 2017), a number of community-based surveys with reasonably large samples reported prevalence rates of 5.2-18.0% for physical abuse (Australian Bureau of Statistics, 2013a; Chu et al., 2013; Mouzos & Makkai, 2004; Price-Robertson et al., 2010; Reeve & van Gool, 2013; Rosenman & Rodgers, 2004), 1.6-4.0% for neglect (both physical and emotional) (Chu et al., 2013; Price-Robertson et al., 2010; Rosenman & Rodgers, 2004), 5.8-17.1% for emotional maltreatment (Chu et al., 2013; Price-Robertson et al., 2010; Rosenman & Rodgers, 2004), 4.3-23.0% for exposure to family violence (Chu et al., 2013; Indermaur, 2001; Price-Robertson et al., 2010; Rosenman & Rodgers, 2004), and 4.0-23.0% for sexual abuse (Australian Bureau of Statistics, 2013a; Mamun et al., 2007; Moore et al., 2010; Najman et al., 2005). There appears to be somewhat higher rates of physical and emotional neglect in the Sydney MAS cohort compared to these Australian statistics, but they are not substantially different from average rates observed across high-income countries (Gilbert et al., 2009). Prevalence estimates may vary due to differences in sampling and definitions of childhood abuse and neglect applied, as well as potential underreporting due to fear and stigma.

Of the several different types of early-life trauma examined in this study, only emotional abuse and Holocaust trauma predicted LLD. While reviews and meta-analyses of long-term health consequences of childhood maltreatment generally suggest experiences of childhood sexual

abuse, physical abuse, emotional abuse, and neglect are all significantly associated with depression (Infurna et al., 2016; Lindert et al., 2014; Norman et al., 2012), Infurna et al. (2016) found that psychological abuse showed the strongest association with depression among the several different forms of childhood maltreatment. Possible explanations for the finding that some types of childhood maltreatment showed no association with LLD in this study include inadequate statistical power due to small sample sizes, as well as potential misclassification due to underreporting given the low prevalence of childhood maltreatment reported in this sample, selection bias as older adults with the most severe depression or high numbers of chronic diseases (a moderator of the childhood maltreatment-LLD link (Comijs et al., 2013)) are unlikely to volunteer for the study, and survivor bias as healthy individuals are more likely to survive into older age. As no further information is available on the specific exposure to the Holocaust, the effect size of the Holocaust trauma-LLD association is possibly underestimated due to misclassification.

In this study, we found no evidence to support the presence of any of the gene-environment interactions examined. Although the specific gene-environment interactions examined here have not been previously investigated in the context of LLD and are exploratory in nature, some of the polymorphisms (for instance, *BDNF* Val66Met and *MTHFR* C677T) have been examined in studies using younger samples. In a meta-analysis of a *BDNF* Val66Met x childhood adversity meta-analysis that pooled studies of child, adolescent, adult and late-life depression together, the authors similarly found no significant interaction between *BDNF* Val66Met and childhood adversity (Hosang et al., 2014). Only one study reported a significant *MTHFR* C677T x childhood trauma interaction in adult depression, but their sample was much smaller and younger compared to our sample (Lok et al., 2013). As power is a common concern in gene-environment interaction studies, further research involving much larger sample sizes is required to elucidate the potential interactions between depression vulnerability genes (including other polymorphisms that are considered to play a role in the stress endocrine system) and exposure to childhood trauma in the risk of developing LLD to ensure there is adequate power to detect an interaction effect (Dick et al., 2015).

A number of neurobiological mechanisms have been proposed to explain the link between early-life trauma and depression, including alterations in HPA axis reactivity, increased neuroinflammation, or volumetric reductions in cortical and limbic regions. Given the important role of the HPA axis in regulating the stress response, a large proportion of the literature has investigated alterations in HPA axis reactivity as a result of childhood abuse and neglect. Heim and colleagues conducted a series of studies investigating the effects of early adverse experiences on neuroendocrine, autonomic and behavioural sensitivity to stress, which are believed to lead to a lower threshold to developing depression (see Heim et al., 2008 for a review). Increased ACTH, cortisol and heart rate responses to stress were observed in individuals with a history of childhood abuse, suggesting that exposure to childhood trauma results in lasting sensitisation of stress responses and dysregulation of the HPA axis, which likely reflect an elevated risk to develop depression in response to subsequent stressors.

Aside from altered HPA axis reactivity, there has also been the suggestion that neuroimmune sensitisation is a prolonged effect of exposure to childhood trauma. It is believed that in addition to neuroinflammatory responses at the time of exposure, childhood trauma may also alter the developmental trajectory of a range of immunological processes, resulting in a heightened immune response to stress later in life. For example, adversity in middle childhood and cumulative adversity from birth were both associated with elevated levels of C-reactive protein (CRP) and interleukin (IL)-6 at ages 10 and 15 (Slopen et al., 2013), and high levels of high-sensitive CRP were observed in adults with current depression and a history of childhood maltreatment (Danese et al., 2008). Pro-inflammatory immune activation has been implicated in a range of neuropsychiatric disorders including depression, with meta-analyses reporting significantly higher concentrations of pro-inflammatory cytokines tumour necrosis factor (TNF)-α and IL-6 in depressed individuals relative to controls (Dowlati et al., 2010), and elevated levels of CRP and IL-6 predicted the development of depressive symptoms (Valkanova et al., 2013).

There is also growing evidence that childhood adversity or trauma leaves persistent epigenetic marks, which alters gene expression in the long term, and may mediate the association between

childhood adversity or trauma and depression. Animal models of early-life stress have found hypomethylation in the enhancer region of the arginine vasopressin (*AVP*) gene in the hypothalamic paraventricular nucleus (Murgatroyd et al., 2009), as well as hypermethylation in regions of the glucocorticoid receptor (*NR3C1*) gene exon 1_7 promoter, accompanied by increased histone H3-K9 acetylation and alterations in nerve growth factor-inducible protein A binding (Weaver et al., 2004). Moreover, a genome-wide transcriptional profiling study of healthy adults showed that individuals with low early-life socioeconomic status had altered neuroendocrine transcriptional activity, including a significant upregulation of genes with response elements for CREB/ATF transcription factors as well as for NF-κB, and a relative downregulation of genes with response elements for the glucocorticoid receptor (Miller et al., 2009b). Collectively, these suggest that the early years of life represents a *sensitive period*, in which the neuroendocrine and neuroimmune systems are shaped by environmental influences, and exposure to early-life adversity leads to the biological programming of a phenotype with characteristics such as exaggerated adrenocortical and neuroinflammatory responses to challenge.

The finding that childhood trauma has enduring effects that persist well into late life underscores the importance of identifying history of childhood trauma in depressed older adults. Earlier studies showed that childhood trauma is not only a significant risk factor for LLD, it is also associated with a more chronic course, greater depression severity and poor response to psychotherapy and/or pharmacotherapy (Nanni et al., 2012; Nelson et al., 2017; Withers et al., 2013). Routine screening of older adults for a history of childhood trauma in primary care may help identify older adults who are at high risk of developing depression, and may also be helpful in treatment selection for this distinct clinical subtype of depression. There is limited research on psychotherapy for older adults with trauma-related mental health issues to date, but a recent systematic review reported evidence from several small studies that exposure-based therapies and eye movement desensitisation and reprocessing appear efficacious in older adults in reducing both post-traumatic stress symptoms and/or depressive symptoms (Dinnen et al., 2015). In addition, mediation studies of childhood trauma and adult depression have shown that general emotion dysregulation partially mediated the association (Hopfinger et al., 2016; Huh et

al., 2017; Schierholz et al., 2016); treatment options that specifically target such deficits may also help alleviate depressive symptoms or prevent their persistence or recurrence in the future.

This chapter shows that experiences of childhood trauma contribute to an increased risk of developing depression in late life. However, no gene-environment interactions were observed. It should be acknowledged that the current study used a relatively small sample, and only a restricted number of SNPs were examined. Measures of early-life trauma were self-reported and retrospective in nature, which may be inaccurate due to recall bias. The literature suggests lasting changes in HPA axis reactivity, neuroimmune response, as well as epigenetic profiles may account for the association between childhood trauma and LLD, but further research is required to elucidate the causal relationships between these alterations and LLD.

7 DNA METHYLATION IN LATE-LIFE DEPRESSION

7.1 Introduction

In Chapters 4 and 5, it was demonstrated that genetic influences explain just over half the variability in the susceptibility to late-life depression (LLD), yet no robust definitive variants have been identified in either candidate gene or genome-wide association studies to date. Twin studies have shown there is some discordance within monozygotic twin pairs (as reviewed in Chapter 4), suggesting that environmental factors also play a role in the aetiology of LLD. One mechanism by which environmental factors may exert their effects on brain development and function is through epigenetic processes. Epigenetic processes contribute to the regulation of gene expression through mechanisms independent of changes in the DNA sequence itself; examples of epigenetic processes include DNA methylation and histone modifications. The most widely studied form of epigenetic regulation is DNA methylation due to its relative stability in somatic cells and ease of measurement (Talens et al., 2010). DNA methylation is catalysed by DNA methyltransferases, which transfer a methyl group from *S*-adenosyl methionine to the fifth carbon of a cytosine residue at the CpG dinucleotide (Jones & Takai, 2001).

There is growing evidence to suggest that DNA methylation play an important role in a range of neuropsychiatric disorders, including depression (Januar et al., 2015b). Early DNA methylation studies of depression mainly assessed methylation changes in the promoter regions of known candidate genes of depression, such as *BDNF* and *SLC6A4*. More recently, epigenome-wide association studies (EWAS) of depression have also been carried out (see Table 7.1 for a summary of EWAS of adult depression with no psychiatric comorbidity). However, published study samples are generally small, various types of tissues have been used, and the findings are mixed or yet to be replicated.

Table 7.1. Summary of DNA methylation studies of depression (including only adult samples with no psychiatric comorbidity).

Study	Sample	Phenotype definition	Tissue	Array/genes investigated	Results	Adjustments for covariates
EWAS						
Byrne et al. (2013)	12 monozygotic (MZ) twin pairs discordant for MDD (31-61 years, 50% female); 12 healthy MZ twin pairs (34-63 years, 50% female) from the Queensland Twin Registry	DSM-IV MDD based on SSAGA/CIDI	White blood cells	450K	↓ global methylation in depressed females than healthy females higher variance in methylation levels in cases than in control co-twins at more than half of the probes	Matched for date of blood sample collection, smoking, alcohol use and history of drug use; all of Northern European ancestry.
Uddin et al. (2011)	33 individuals with lifetime history of depression (43.5±11.9 years, 70% female) and 67 healthy controls (46.2±18.7 years, 55% female) from the Detroit Neighbourhood Health Study	Depressive symptoms: PHQ-9 Lifetime depression: depressed mood or anhedonia, and ≥1 other symptom for ≥2 weeks and/or suicidal or self-harm thoughts	Blood	27K	the depressed group had fewer uniquely methylated genes compared to controls *IL-6* methylation inversely correlated with serum IL-6 and CRP	Groups showed no significant differences in genetic background, education, smoking, alcohol consumption, medication use, PBMC count, or serum IL-6 or CRP levels.

Study	Sample	Phenotype definition	Tissue	Array/genes investigated	Results	Adjustments for covariates
Candidate gene						
D'Addario et al. (2013)	41 MDD patients and 44 healthy controls	DSM-IV MDD based on SCID	PBMCs	*BDNF* promoter	↑ *BDNF* promoter methylation in MDD compared to controls	Matched for age.
Fuchikami et al. (2011)	20 MDD patients (45.6±12.5 years, 60% female) and 18 healthy controls (42.3±9.6 years, 80% female)	DSM-IV MDD based on Japanese version of MINI	Blood	*BDNF* promoter	methylation profiles of CpG I but not CpG IV of the *BDNF* gene can be used to accurately distinguish between MDD patients and healthy controls.	N/A
Na et al. (2014)	45 MDD patients (41.6±11.8 years, 76% female) and 72 healthy controls (40.7±14.2 years, 71% female)	DSM-IV MDD based on Korean version of SCID	Blood	*NR3C1*	↓ methylation at CpG 3 and CpG 4 of *NR3C1* in MDD patients compared to healthy controls.	Matched for age and sex.
Melas et al. (2013)	*MAOA* study: 82 MDD patients (23-74 years, 100% female) and 92 controls (21-74 years, 100% female) *NR3C1* study: 93 MDD patients (23-74 years, 100% female) and 83 healthy controls (21-74 years, 100% female)	DSM-IV MDD based on MDI	Saliva	*MAOA*	↓ mean *MAOA* methylation and *MAOA* CpG8 methylation	N/A

Study	Sample	Phenotype definition	Tissue	Array/genes investigated	Results	Adjustments for covariates
Iga et al. (2016)	28 MDD patients (45.0±13.1 years, 71% female) and 29 healthy controls (42.2±12.1 years, 72% female)	DSM-IV MDD	Leukocytes	*SLC6A4* promoter	↑ mean *SLC6A4* promoter methylation in MDD patients compared to healthy controls, but no significant differences at any single CpG site. Dose-dependent relationship between *SLC6A4* 5-HTTLPR genotype and mean methylation level in patients.	N/A
Okada et al. (2014)	50 MDD patients (40.3±10.3 years, 46% female) and 50 healthy controls (40.3±10.5 years, 46% female)	DSM-IV MDD based on Japanese version of MINI	Blood	*SLC6A4* promoter	Unable to distinguish between MDD patients and healthy controls using *SLC6A4* promoter methylation	N/A

Abbreviations: 450K – Illumina HumanMethylation 450K BeadChip; 27K – Illumina HumanMethylation 27K BeadChip; CIDI – Composite International Diagnostic Interview; CRP – C-reactive protein; DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, 4th edition; IL – interleukin; MDD – major depressive disorder; MDI – Major Depression Inventory; MINI – Mini International Neuropsychiatric Interview; PHQ – Patient Health Questionnaire; SCID – Structured Clinical Interview for DSM-IV Axis I Disorders; SSAGA – Semi-Structured Assessment for the Genetics of Alcoholism

Thus far, only two studies examining DNA methylation in LLD have been published, both of which employed the candidate gene approach and investigated *BDNF* promoter methylation in LLD (Januar et al., 2015a; Kang et al., 2015). Earlier studies reported that increased *BDNF* promoter methylation is associated with reduced synthesis of BDNF in mouse cortical neurons (Martinowich et al., 2003), and with depression and suicidal ideation in younger adult samples (Fuchikami et al., 2011; Kang et al., 2013). With regard to LLD, Kang et al. (2015) investigated the role of the *BDNF* Val66Met polymorphism (rs6265) as well as *BDNF* promoter methylation in blood in LLD, and higher *BDNF* promoter methylation at CpG site 9 was associated with both prevalence at baseline and incidence at the 2-year follow-up. Higher *BDNF* promoter methylation at CpG site 9 was also associated with greater depression severity as assessed on the GDS. However, no significant genotype x methylation interaction effects on the prevalence or incidence of LLD were observed. Using buccal DNA methylation, Januar et al. (2015a) showed that increased methylation at the CpG unit 3.4.5 in *BDNF* promoter I and at CpG site 3 in promoter IV were associated with baseline depression as well as chronic depression (defined as being depressed at three or more of the assessments over 12 years of follow-up). However, in contrast to the non-significant genotype x methylation interaction found in Kang et al. (2015), Januar et al. (2015a) reported that two SNPs (rs6265 and rs7103411) modified the depression-methylation association.

Since no epigenome-wide association studies of LLD have been conducted, the aim of this chapter is to examine epigenetic variation associated with LLD using blood DNA methylation from a sample of older monozygotic (MZ) twin pairs. A secondary analysis was conducted using the discordant MZ twin design as it offers the advantage of controlling for a range of potential confounding factors, such as genetic background, age, sex, maternal influences, early environmental experiences to some extent as well as population cohort effects (Bell & Spector, 2011; van Dongen et al., 2012).

7.2 Methods

7.2.1 Participants

The sample comprised participants from the Older Australian Twins Study (OATS) (Sachdev et al., 2009). Monozygotic twin pairs with both DNA methylation and depression data in Wave 1 of OATS were included. Twin pairs who had their blood samples processed in different states were excluded, and a twin pair with a known chromosomal rearrangement was also excluded.

7.2.2 Measures

Depression status was defined by a composite of the 15-item Geriatric Depression Scale (GDS-15) (Yesavage & Sheikh, 1986) total score (using a cut-off of six or above) and self-reported current antidepressant use. Smoking status, drinking status and physical activity were included as covariates. Refer to Chapter 3 for more details.

7.2.3 Procedures

DNA was extracted from peripheral blood samples using either the Qiagen Autopure (Qiagen, Valencia, CA, USA) or a proteinase K salting out method was used. Within experiments, co-twins were randomised across the arrays. DNA methylation status was examined using the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, CA, USA). Raw intensity data were background corrected and methylation beta values (ranging from 0-1) were generated using the R *minfi* package (Aryee et al., 2014). Functional normalisation was used. Sex chromosome probes, probes containing SNPs, cross-reactive probes as well as probes not detected in all samples were excluded from the analysis (Chen et al., 2013a). After these quality control (QC) procedures there were 420,982 out of 485,764 probes remaining and the distribution of probe types before and after QC is presented in Table 7.2 below. White blood cell composition was estimated using the method described in Houseman et al. (2012), implemented in *minfi*.

Table 7.2. Distribution of probe types before and after quality control.

Annotation	Before quality control (Sandoval et al., 2011)	After quality control
Island	150,254 (30.9%)	135,919 (32.3%)
Shore	112,072 (23.1%)	99,109 (23.5%)
Shelf	47,161 (9.7%)	39,189 (9.3%)
Open sea	176,277 (36.3%)	146,765 (34.9%)
Total	485,764	420,982

7.2.4 Epigenome-wide association study

An EWAS was conducted using the beta values on each probe as the dependent variable, and depression status as the independent variable. After regressing out the first two principal components of white blood cell composition counts and the first four principal components of beta values to adjust for cell composition and batch effects respectively, the EWAS was performed using generalised estimating equations in the full MZ twin sample to account for the twin structure and using simple *t*-tests in the discordant MZ twin sample. Age, sex, smoking status, drinking status and physical activity were included as covariates in the EWAS using the full MZ twin sample. The Benjamini-Hochberg method was applied in both analyses to account for multiple testing and a false discovery rate of $p < 0.05$ was considered statistically significant.

7.2.5 Pathway analysis

In order to determine whether the identified differentially methylated probes were in or near genes that clustered together functionally, over-representation enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) Biological Process categories were carried out using the WebGestalt 2017 (WEB-based Gene SeT AnaLysis Toolkit; available at http://www.webgestalt.org). WebGestalt uses Fisher's exact test to evaluate over- or under-representation of genes in a gene set of interest compared to a reference gene set. The target gene set comprised unique genes associated with the top 1,000 probes ranked in ascending order of the unadjusted *p* value, where there is more than one gene associated with a probe only the closest one is included. All genes mapped to Illumina Human Methylation 450K probes were used as the reference set (n = 19,211 unique genes). Of those 19,211 genes, 6,242 are annotated to KEGG pathways (release Oct 1, 2016) and 14,613 are annotated to GO Biological

Process categories (release Oct 24, 2016). The Benjamini-Hochberg method was used to correct for multiple testing and false discovery rate of $p < 0.05$ was considered statistically significant.

7.2.6 Blood-brain DNA methylation comparison

While it is known that DNA methylation is largely tissue-specific, significant correlations between DNA methylation measured in blood, cortex and cerebellum have been observed within individuals for a subset of CpG sites (Hannon et al., 2015). For the differentially methylated probes identified in this study, an indication of their likely relevance was established by obtaining blood-brain methylation correlations using the Blood Brain DNA Methylation Comparison Tool (available at http://epigenetics.iop.kcl.ac.uk/bloodbrain/). The tool provides correlations between DNA methylation in blood with four brain regions – prefrontal cortex (n = 74), entorhinal cortex (n = 71), superior temporal gyrus (n = 75) and cerebellum (n = 71) – from matched samples for all probes on the Illumina 450K array. Pearson's correlations with a $p < 0.05$ were considered statistically significant.

7.3 Results

7.3.1 Participants

A total of 97 MZ twin pairs (7 pairs concordant with depression, 17 pairs discordant for depression, and 73 healthy control pairs) were included in this study. All participants were Caucasian. Detailed sample characteristics are presented in Table 7.3.

Table 7.3. Sample demographics (*M* ± *SD* or *n* (%)).

	Concordant (*n* = 7 pairs)	Discordant (*n* = 17 pairs)	Healthy controls (*n* = 73 pairs)	*P*[a]
Age, years	67.0 ± 2.1	70.5 ± 6.6	71.5 ± 5.9	0.15
Sex, female	12 (85.7)	22 (64.7)	43 (58.9)	1.18e-15
GDS-15 total	2.6 ± 3.1[b]	3.0 ± 2.7	1.2 ± 1.0	1.56e-08
Smoker status[c]				0.10
- non-smoker	8 (57.1)	17 (50.0)	95 (65.1)	
- past smoker	4 (28.6)	11 (32.4)	45 (30.8)	
- current smoker	2 (14.3)	5 (14.7)	5 (3.4)	
Drinker status[c]				0.27
- non-drinker	2 (14.3)	1 (2.9)	15 (10.3)	
- past drinker	2 (14.3)	1 (2.9)	14 (9.6)	
- current drinker	10 (71.4)	31 (91.2)	117 (80.1)	
Physical activity, min	522.9 ± 418.0	366.2 ± 365.7	574.3 ± 534.5	0.10

[a] Group differences were tested using ANOVA for continuous variables and Fisher's exact test for categorical variables.
[b] As depression caseness was defined as a GDS-15 score of 6 or above or self-reported antidepressant use, the depressed group includes participants with depression scores below the cut-off. In this study, six out of the seven concordant twin pairs were on antidepressants, and most of them had subthreshold GDS-15 scores.
[c] Numbers do not add up to 100% due to missing data.

7.3.2 Epigenome-wide association studies

7.3.2.1 Full MZ twin sample

When the full sample was used for the analysis, a total of 69 significantly differentially methylated probes were identified after correction for multiple testing (see Figure 7.1 and Table 7.4). The distribution of probes was as follows: 23 (33.3%) are located in CpG islands, 17 (24.6%) in CpG shores, and 5 (7.2%) in CpG shelves. In terms of the functional genomic distribution, 25 (36.2%) of the probes were located in the promoter region of a gene, defined as the combined region of within 1,500bp or within 200bp upstream of the transcription start site and in the 5'-untranslated region, one in the 1st exon (1.4%), 21 (30.4%) within the gene, 4 (5.8%) in the 3'-untranslated region, and the remaining 18 (26.1%) are in intergenic regions. The significant probes did not appear to cluster in any particular chromosomal location. Of note, none of the CpG sites associated with known candidate genes of depression (such as *BDNF* or *SLC6A4*) reached statistical significance.

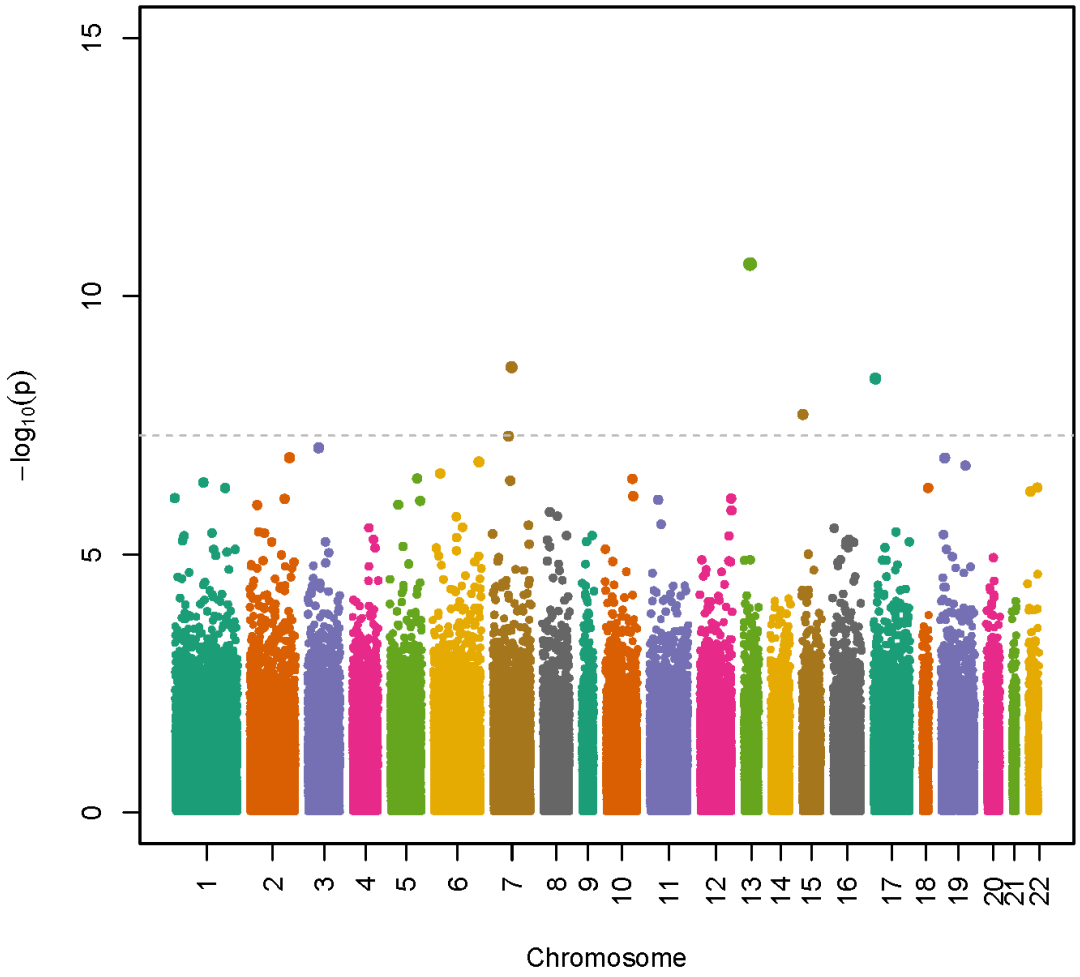

Figure 7.1. Manhattan plot of unadjusted *p*-values of all probes tested in the EWAS using the full MZ twin sample.

Table 7.4. Details of the significantly differentially methylated probes (n = 69) identified in the full twin sample EWAS.

Probe	*β*	*FDR*	Coordinates (hg19)	Gene symbol (UCSC)	Gene region feature category (UCSC)	Relationship to CpG island
cg00247571	-0.037	1.01E-05	Chr13:50,707,065			N_Shore
cg22525688	-0.031	5.03E-04	Chr7:74,001,908	*GTF2IRD1*	Body	
cg27406664	0.017	5.59E-04	Chr17:2,294,951	*MNT*	Body	N_Shore
cg23052037	0.019	2.07E-03	Chr15:26,229,322			
cg16703135	-0.041	4.39E-03	Chr7:55,517,132			Island
cg16871520	0.012	5.94E-03	Chr3:52,442,033	*BAP1*	Body	N_Shore
cg03047616	0.014	7.04E-03	Chr2:230,281,966	*DNER*	Body	
cg09422806	0.010	7.04E-03	Chr19:3,364,015	*NFIC*	Body	N_Shelf
cg23216724	-0.028	7.46E-03	Chr6:167,571,584	*GPR31*	TSS1500	
cg09542063	-0.006	8.05E-03	Chr19:45,349,057	*PVRL2 [NECTIN2]*	TSS1500	N_Shore
cg06373574	-0.019	1.05E-02	Chr6:26,569,596			
cg18994744	0.020	1.13E-02	Chr5:166,802,318	*ODZ2 [TENM2]*	Body	
cg26282761	0.027	1.13E-02	Chr10:128,193,219	*C10orf90*	Body	N_Shore
cg19240483	0.009	1.14E-02	Chr7:72,985,061	*TBL2*	Body	Island
cg24596244	0.014	1.15E-02	Chr1:59,920,114	*FGGY*	Body	
cg02122610	-0.020	1.23E-02	Chr22:47,022,418	*GRAMD4*	TSS1500	N_Shore
cg25916714	0.025	1.23E-02	Chr18:74,691,269	*MBP*	3'UTR	Island
cg00115313	-0.022	1.23E-02	Chr1:193,029,121	*TROVE2; UCHL5*	(5'UTR & 1stExon); TSS1500	Island
cg11792483	0.004	1.36E-02	Chr22:26,824,763	*ASPHD2*	TSS1500	N_Shore
cg13303464	0.010	1.50E-02	Chr10:130,844,209			
cg02138082	0.023	1.50E-02	Chr1:978,573	*AGRN*	Body	Island
cg08130329	0.018	1.50E-02	Chr12:132,946,585			
cg12870876	-0.022	1.50E-02	Chr2:203,736,356	*ICA1L*	(5'UTR, 1stExon & TSS200)	Island
cg12196305	-0.022	1.51E-02	Chr11:17,752,634			N_Shelf

Probe	β	*FDR*	Coordinates (hg19)	Gene symbol (UCSC)	Gene region feature category (UCSC)	Relationship to CpG island
cg03712341	-0.013	1.52E-02	Chr5:177,659,383	*AGXT2L2 [PHYKPL]*	Body	Island
cg12181407	-0.022	1.70E-02	Chr5:54,052,513			
cg17284481	-0.017	1.70E-02	Chr2:30,670,304	*LCLAT1*	5'UTR	Island
cg20109840	-0.009	2.09E-02	Chr12:133,019,038			Island
cg12564175	0.015	2.16E-02	Chr8:27,630,986	*ESCO2; CCDC25*	TSS1500; TSS1500	N_Shore
cg22863920	-0.009	2.54E-02	Chr8:72,470,832			S_Shore
cg07500019	0.009	2.54E-02	Chr6:33,281,113	*TAPBP*	Body	Island
cg22975913	-0.015	3.45E-02	Chr11:32,457,727	*WIT1 [WT1-AS]; WT1*	Body; TSS1500	N_Shore
cg19256125	-0.012	3.51E-02	Chr7:155,249,528	*EN2*	TSS1500	Island
cg01761966	-0.022	3.67E-02	Chr6:52,173,014			S_Shore
cg13169863	-0.009	3.67E-02	Chr4:108,783,348	*SGMS2*	5'UTR	
cg10224476	0.019	3.67E-02	Chr16:1,139,166	*C1QTNF8*	3'UTR	N_Shore
cg06043190	0.024	4.07E-02	Chr2:42,396,882	*EML4*	Body	Island
cg10820158	0.021	4.07E-02	Chr17:53,810,813	*TMEM100*	TSS1500	
cg23202177	-0.047	4.07E-02	Chr1:116,383,238	*NHLH2*	(5'UTR & 1stExon)	S_Shore
cg06552799	-0.007	4.07E-02	Chr2:71,559,058	*ZNF638; ZNF638*	5'UTR; 5'UTR	Island
cg25001831	0.016	4.07E-02	Chr7:622,212	*PRKAR1B*	Body	
cg15164761	-0.007	4.07E-02	Chr19:2,255,496	*JSRP1*	TSS200	S_Shore
cg00595039	0.010	4.07E-02	Chr8:143,460,209	*TSNARE1*	5'UTR	
cg13708759	0.015	4.07E-02	Chr9:138,585,730	*SOHLH1*	(3'UTR & Body)	
cg07159230	-0.013	4.07E-02	Chr1:10,731,250	*CASZ1*	Body	
cg02382037	-0.032	4.07E-02	Chr12:130,823,570	*PIWIL1*	5'UTR	Island
cg06289138	-0.040	4.27E-02	Chr6:33,561,186	*C6orf227 [LINC00336]*	TSS200	Island
cg07717381	-0.035	4.35E-02	Chr16:56,716,320	*MT1X*	TSS200	Island
cg09735140	0.011	4.35E-02	Chr4:152,330,774	*FAM160A1*	5'UTR	Island

Probe	*β*	*FDR*	Coordinates (hg19)	Gene symbol (UCSC)	Gene region feature category (UCSC)	Relationship to CpG island
cg07512997	0.018	4.35E-02	Chr8:21,201,211			
cg15639718	0.021	4.35E-02	Chr1:8,361,284			
cg05167429	0.030	4.35E-02	Chr16:33,854,086			S_Shore
cg14521115	0.013	4.35E-02	Chr9:104,214,581			S_Shelf
cg13258195	0.010	4.35E-02	Chr17:80,300,821			N_Shelf
cg16263825	0.023	4.35E-02	Chr3:114,866,835	*ZBTB20*	TSS1500	Island
cg09982942	-0.047	4.35E-02	Chr2:112,898,400	*FBLN7*	Body	S_Shore
cg11231977	-0.006	4.35E-02	Chr16:75,467,423	*CFDP1*	TSS200	Island
cg26561212	0.023	4.66E-02	Chr7:157,255,200			
cg04374711	-0.016	4.82E-02	Chr16:31,008,960	*STX1B*	Body	Island
cg16533147	-0.017	4.93E-02	Chr5:87,963,342	*LOC645323 [LINC00461]*	Body	
cg07150473	0.028	4.93E-02	Chr8:27,338,183	*CHRNA2*	TSS1500	
cg24174232	0.016	4.93E-02	Chr17:29,818,175	*RAB11FIP4*	Body	S_Shelf
cg09621330	-0.062	4.93E-02	Chr16:54,685,651			
cg11836444	-0.007	4.93E-02	Chr4:166,131,163	*KLHL2*	(TSS200 & Body)	S_Shore
cg20462795	0.026	4.93E-02	Chr6:10,981,184	*ELOVL2*	3'UTR	
cg11740194	-0.009	4.96E-02	Chr10:466,232	*DIP2C*	Body	Island
cg06095777	-0.016	4.96E-02	Chr1:145,714,010	*CD160*	5'UTR	Island
cg19571127	-0.012	4.96E-02	Chr19:4,867,897	*PLIN3*	TSS200	
cg05051043	0.029	4.96E-02	Chr1:234,040,765	*SLC35F3*	(1stExon & 5'UTR)	Island

Abbreviations: TSS200 – 0-200 bases upstream of the transcriptional start site (TSS); TSS1500 – 200-1500 bases upstream of the TSS; 5'UTR – the 5' untranslated region, between the TSS and the ATG start site; body – between the ATG and stop codon, 3'UTR – the 3' untranslated region, between the stop codon and poly A signal

7.3.2.2 Discordant MZ twins

The *t*-test analysis of the 17 discordant MZ twin pairs was unable to identify any differentially methylated probes of statistical significance after correction for multiple testing.

7.3.3 Pathway analysis

From the model that used the entire MZ twins sample, 715 unique genes associated with the 1,000 top-ranked probes were entered into WebGestalt as the target list for analysis. None of the pathways remained statistically significant after correction for multiple testing; the top 10 enriched pathways are listed in Table 7.5.

Table 7.5. Top 10 enriched KEGG pathways.

KEGG pathway	Fold enrichment	*p*	*FDR*
hsa04211 Longevity regulating pathway	+2.90	2.17E-03	4.19E-01
hsa04974 Protein digestion and absorption	+3.01	2.76E-03	4.19E-01
hsa05202 Transcription misregulation in cancer	+2.20	4.38E-03	4.42E-01
hsa04725 Cholinergic synapse	+2.44	7.58E-03	5.12E-01
hsa04915 Oestrogen signalling pathway	+2.47	1.02E-02	5.12E-01
hsa04722 Neurotrophin signalling pathway	+2.29	1.16E-02	5.12E-01
hsa04152 AMPK signalling pathway	+2.21	1.46E-02	5.12E-01
hsa04912 GnRH signalling pathway	+2.43	1.66E-02	5.12E-01
hsa04726 Serotonergic synapse	+2.20	2.09E-02	5.12E-01
hsa04730 Long-term depression	+2.70	2.27E-02	5.12E-01

A total of 13 GO categories were statistically significant after correction for multiple testing, most of which concern developmental processes (see Table 7.6).

Table 7.6. Significantly enriched GO biological processes.

GO biological process	Fold enrichment	*p*	*FDR*
GO:0009887 animal organ morphogenesis	+1.90	4.57E-07	2.58E-03
GO:0072359 circulatory system development	+1.91	7.28E-07	2.58E-03
GO:0007507 heart development	+2.29	9.32E-07	2.58E-03
GO:0048646 anatomical structure formation involved in morphogenesis	+1.88	1.24E-06	2.58E-03
GO:0048562 embryonic organ morphogenesis	+2.69	2.99E-06	4.99E-03
GO:0007423 sensory organ development	+2.15	9.80E-06	1.24E-02
GO:0007417 central nervous system development	+1.81	1.27E-05	1.24E-02
GO:0090596 sensory organ morphogenesis	+2.72	1.38E-05	1.24E-02
GO:0009790 embryo development	+1.77	1.41E-05	1.24E-02
GO:0048568 embryonic organ development	+2.22	1.57E-05	1.24E-02
GO:0048598 embryonic morphogenesis	+2.03	1.64E-05	1.24E-02
GO:0022008 neurogenesis	+1.59	2.67E-05	1.77E-02
GO:0048699 generation of neurons	+1.61	2.76E-05	1.77E-02

Since the discordant MZ twins EWAS did not identify any significant probes, a pathway analysis was not performed using those results.

7.3.4 Blood-brain DNA methylation correlations

Blood-brain methylation correlations were investigated using the Blood Brain DNA Methylation Comparison Tool for the 69 significant probes, and it was discovered that 29 of the probes showed significant blood-brain methylation correlation in at least one of the four brain regions examined (see Table 7.7). The comparison tool, however, does not have information on methylation in the hippocampus, which is believed to play an important role in the pathophysiology of depression (MacQueen & Frodl, 2010). Interestingly, four probes showed significant blood-brain methylation correlations in all four brain regions, three of which mapped to a nearby gene or were located within the body of a gene.

Table 7.7. Correlations of DNA methylation levels in blood with four brain regions.

Probe	Prefrontal cortex	Entorhinal cortex	Superior temporal gyrus	Cerebellum
cg00247571	-0.08	-0.03	-0.23	-0.04
cg22525688	-0.01	-0.17	0.14	0.14
cg27406664	0.18	0.18	**0.24**	**0.44**
cg23052037	-0.11	0.10	0.06	-0.07
cg16703135	**0.33**	**0.28**	**0.27**	0.14
cg16871520	0.09	0.04	-0.02	**-0.25**
cg03047616	0.09	0.10	-0.02	0.07
cg09422806	**0.26**	0.06	0.05	-0.01
cg23216724	-0.06	-0.11	0.16	-0.17
cg09542063	-0.13	-0.02	0.18	-0.01
cg06373574	0.16	**0.52**	0.23	0.12
cg18994744	**0.45**	0.19	**0.25**	0.18
cg26282761	0.13	**0.38**	0.13	0.10
cg19240483	0.18	0.08	0.21	0.01
cg24596244	0.11	-0.02	0.09	-0.06
cg02122610	-0.04	-0.09	-0.04	0.16
cg25916714	-0.03	0.05	-0.17	-0.15
cg00115313	0.13	0.01	0.21	-0.15
cg11792483	-0.01	0.17	0.01	-0.06
cg13303464	-0.09	-0.08	0.14	-0.09
cg02138082	**0.70**	**0.72**	**0.64**	**0.70**
cg08130329	**0.34**	0.17	-0.01	-0.02
cg12870876	0.09	0.12	0.00	0.10
cg12196305	0.05	**0.27**	0.04	0.09
cg03712341	-0.05	-0.02	0.03	-0.17
cg12181407	0.11	0.07	**0.25**	0.19
cg17284481	**0.31**	0.08	**0.65**	-0.03
cg20109840	0.11	0.15	**0.36**	-0.01
cg12564175	**0.64**	**0.46**	**0.62**	**0.64**
cg22863920	**0.42**	0.13	0.21	0.09
cg07500019	-0.02	0.03	-0.22	0.05
cg22975913	0.02	0.00	**0.24**	-0.08
cg19256125	0.01	0.19	**0.29**	0.08
cg01761966	0.09	-0.09	0.10	0.09
cg13169863	0.04	-0.05	0.12	0.12
cg10224476	0.05	0.17	0.16	0.03
cg06043190	-0.04	0.07	-0.13	-0.21
cg10820158	0.11	**0.28**	0.11	0.20
cg23202177	0.17	**0.32**	**0.36**	0.10
cg06552799	0.09	0.18	-0.21	-0.06
cg25001831	0.07	-0.12	-0.12	-0.02
cg15164761	0.15	0.08	0.09	-0.01
cg00595039	0.09	0.04	-0.19	0.05

Probe	Prefrontal cortex	Entorhinal cortex	Superior temporal gyrus	Cerebellum
cg13708759	-0.01	0.04	**0.23**	0.15
cg07159230	-0.02	0.23	-0.07	0.08
cg02382037	**0.41**	**0.59**	**0.43**	0.22
cg06289138	0.19	0.07	**0.28**	-0.01
cg07717381	-0.03	0.07	-0.09	-0.09
cg09735140	-0.11	-0.09	-0.14	**-0.23**
cg07512997	0.02	**0.26**	**0.31**	0.08
cg15639718	-0.06	-0.03	-0.02	0.05
cg05167429	**0.33**	**0.45**	**0.39**	**0.24**
cg14521115	**0.26**	-0.03	-0.15	0.11
cg13258195	0.03	-0.03	0.08	-0.11
cg16263825	0.18	0.18	0.06	0.18
cg09982942	**0.50**	**0.47**	**0.50**	**0.43**
cg11231977	-0.08	0.18	-0.04	-0.22
cg26561212	-0.02	0.13	0.03	**0.29**
cg04374711	-0.10	-0.09	0.05	-0.11
cg16533147	0.23	0.05	-0.02	0.22
cg07150473	-0.11	0.07	0.05	0.05
cg24174232	0.07	0.15	-0.02	0.18
cg09621330	-0.13	0.11	0.11	-0.18
cg11836444	0.22	**0.24**	**0.23**	**0.69**
cg20462795	-0.07	-0.20	0.04	0.01
cg11740194	0.06	-0.08	0.10	0.09
cg06095777	0.16	0.23	0.00	0.13
cg19571127	0.04	0.13	-0.05	-0.14
cg05051043	0.04	0.06	0.17	0.02

Note: Correlations significant at $p < 0.05$ are indicated in bold.

7.4 Discussion

In this chapter, an EWAS of LLD was conducted for the first time using blood DNA methylation from a sample of older MZ twins. The present study is the first epigenome-wide study of LLD. When using the full sample of MZ twin pairs, a total of 69 significantly differentially methylated probes associated with LLD were identified. While the discordant MZ twins design is the ideal design to be used in investigating DNA methylation differences associated with LLD, the sample of 17 discordant MZ twin pairs proved to be inadequately powered to detect differentially methylated probes, as none of the probes survived correction for multiple testing.

The two earlier candidate gene methylation studies of LLD have both focused on the promoter region of *BDNF* (Januar et al., 2015a; Kang et al., 2015), based on the understanding that methylation in the promoter region is associated with repression of transcriptional activity (Jones, 2012; Moore et al., 2013). However, only around a third of the probes identified in the present study are located in the promoter region of a gene. More recent research paints a more complex picture by showing that methylation in other locations may also have regulatory effects on transcriptional activity. For instance, gene body methylation appears to be positively correlated with gene expression in proliferating cells (Aran et al., 2011; Hellman & Chess, 2007) but not others (Aran et al., 2011; Guo et al., 2011; Oh et al., 2013). It has also been proposed that gene body methylation plays a role in the prevention of aberrant transcription (Neri et al., 2017) and the modulation of alternative splicing (Maunakea et al., 2013). Moreover, while the first exon is located downstream of the transcription start site, Brenet et al. (2011) showed that methylation of the first exon has similar effects to methylation in the promoter region, which results in transcriptional repression. About a quarter of the identified probes are not located in the vicinity of any genes, and the function of intergenic DNA methylation is yet undefined.

Among the genes associated with the significant probes, *ZBTB20* has been previously implicated in an EWAS of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) MDD using whole blood methylation in a sample of adult MZ twin pairs (age range: 23-73 years) (Davies et al., 2014). The study also examined gene expression in post-mortem brain samples and found that *ZBTB20* is co-expressed with a unique set of genes in the hippocampus. Mouse studies suggest the *Zbtb20* gene plays essential roles in the developmental specification of CA1 pyramidal neurons in the developing hippocampus (Nielsen et al., 2010; Nielsen et al., 2014; Xie et al., 2010) as well as in the regulation of pituitary development (Cao et al., 2016), and regulates memory formation and synaptic plasticity in mature CA1 neurons (Ren et al., 2012). Moreover, ectopic expression of *Zbtb20* appears to lead to malformations in cortical development that are linked to behavioural abnormalities (Nielsen et al., 2007).

A number of the other significant probes are located in the proximity of genes that have previously been linked to other neuropsychiatric or neurodegenerative disorders, for example *TSNARE1* and *NECTIN2*.

The *TSNARE1* gene is located at 8q24.3 and has been repeatedly identified as associated with susceptibility to schizophrenia in genome-wide association studies (Gu et al., 2015; Ripke et al., 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Sleiman et al., 2013) and is involved in synaptic vesicular trafficking. While there is currently limited knowledge of its function, a recent zebrafish model has shown that overexpression of *tsnare1* leads to a significant increase in cell proliferation as well as apoptosis (Fromer et al., 2016), suggesting that it may be involved in the regulation of neurogenesis.

Significant signals in or near the *NECTIN2* gene (19q13.32) have been reported in a number of genome-wide association studies of dementia (Abraham et al., 2008; Ferrari et al., 2017; Miyashita et al., 2013; Pérez-Palma et al., 2014), for which depression is a risk factor and a highly comorbid disorder. It has also been associated with neuropathological features of AD like neurofibrillary tangles and neuritic plaques (Beecham et al., 2014) as well as biomarkers of AD such as beta-amyloid, tau and phosphorylated tau levels in cerebrospinal fluid (Cruchaga et al., 2013; Deming et al., 2017). Significant differential expression of *NECTIN2* has also been observed between individuals with idiopathic Parkinson's disease and controls (Infante et al., 2015). A recent study showed that the *Nectin-2* was expressed in neurons and astrocytes in the mouse brain, and *Nectin-2* -/- mouse brains showed age-related atrophy in both white and grey matter, increased apoptosis, as well as degeneration of perivascular astrocytic endfoot processes (Miyata et al., 2016).

No KEGG pathways were significantly over-represented. However, despite the lack of significance, most of the top-ranked enriched pathways are related to intracellular signalling and synaptic transmission, which are thought to be disrupted in depression. Interestingly, the set of significantly over-represented gene ontology categories were all developmental processes, including central nervous system development and neurogenesis, which is a finding similar to what was observed in an earlier genome-wide DNA methylation scan of major depressive

disorder (MDD) in post-mortem brains despite using a different methodology and a different tissue (Sabunciyan et al., 2012). This provides some evidence in support of the neurotrophic hypothesis of depression which postulates that reduced neurogenesis plays a critical role in the aetiology of depression.

Upon examining blood-brain correlations of methylation levels, less than half of the significant probes showed significant correlations in at least one of the brain regions examined. It should, however, be noted that methylation has been measured only in four brain regions (prefrontal cortex, entorhinal cortex, superior temporal gyrus and the cerebellum), and it cannot be ruled out that blood methylation may be significantly correlated with methylation in other regions of the brain, particularly the hippocampus in which reduced neurogenesis is implicated in a range of neuropsychiatric and neurodegenerative disorders. In fact, a number of cross-tissue studies have shown while DNA methylation is tissue-specific, a small subset of CpG sites show statistically significant blood-brain correlations (Davies et al., 2012; Hannon et al., 2015; Masliah et al., 2013; Walton et al., 2016). Although the majority of CpG sites do not show significant blood-brain correlations and may only provide limited information on the pathogenesis of neuropsychiatric disorders, methylation signatures in blood may still be informative as diagnostic or prognostic biomarkers for neuropsychiatric disorders (Aberg et al., 2013; Hannon et al., 2015). Furthermore, while neuropsychiatric disorders clearly involve structural and functional alterations in the brain, it is possible that disruptions of processes and pathways beyond the brain may also play mechanistic roles (Bakulski et al., 2016), such as dysfunctions of the immune system and the gut-brain axis.

Several limitations should be noted. First, the present study is a preliminary study in a relatively small sample; replication studies in independent samples, ideally large-scale multi-site studies and/or conducted using the discordant MZ twin design to control for potential confounding effects such as genetic variation, are necessary to confirm the observed associations of the identified sites with LLD. Second, the majority of significant probes were not located in the promoter region and the effects of methylation at these sites are unclear, and the pathway analysis also does not take the subset of probes not located in the vicinity of any genes into

account. Finally, the present study does not differentiate between early-onset recurrent depression and late-onset depression, which could have confounding effects as the literature suggests the two subtypes may be clinically distinct, as discussed in Chapter 2. Besides addressing the aforementioned limitations, future studies should also examine DNA methylation profiles longitudinally to identify potential diagnostic or prognostic marker(s) for LLD.

In summary, this chapter presents the first DNA methylation study of LLD using a data-driven approach, and a number of significantly differentially methylated probes were identified. Several probes were located in or near genes that have been previously implicated in neuropsychiatric or neurodegenerative disorders, and their potential role in the pathogenesis of LLD was discussed.

8 GENERAL DISCUSSION AND CONCLUSIONS

8.1 Review of thesis objective and aims

In an era of global population ageing, the increasing prevalence of mental and physical disorders in older adults represents a significant public health concern. In particular, depressive syndromes are among the leading causes of disability in populations aged 60 years and over, and are associated with higher rates of morbidity and mortality as well as increased health care costs (Blazer, 2003; Bock et al., 2016; World Health Organization, 2015). Late-life depression (LLD) is believed to involve a complex interplay of biological and environmental influences; however, there is currently limited understanding on its mechanistic underpinnings. In Chapter 1, it was argued that a closer examination of the biological and environmental influences on LLD could obtain new insights into the neurobiological pathways involved in the pathophysiology of LLD, and may help identify at-risk subpopulations as potential targets for preventive strategies to reduce the disease burden.

This thesis set out to examine the genetic, environmental and epigenetic influences on depression in older adults, using samples from longitudinal ageing cohort studies, i.e. the Older Australian Twins Study (OATS) (Sachdev et al., 2009) and the Sydney Memory and Ageing Study (MAS) (Sachdev et al., 2010; Tsang et al., 2013). The specific research aims were (i) to examine the relative contribution of genetic and environmental influences on LLD and associated phenotypes, (ii) to review the current literature for susceptibility genetic polymorphisms for LLD or depressive symptoms, (iii) to investigate the role of early-life trauma in LLD, and (iv) to explore the role of DNA methylation in LLD. These research aims were addressed in the four studies reported in the preceding chapters; the key findings of these studies will be summarised below.

8.2 Summary of key findings

8.2.1 Genetic epidemiology of late-life depression and associated phenotypes

Chapter 4 addressed the first research aim and examined the sources of variation in LLD. Using a relatively broad definition of depression (individuals who scored a six or above on the 15-item Geriatric Depression Scale (GDS-15) or self-reported antidepressant use were classified as depressed), the study demonstrated that more than half of the variance in LLD could be attributed to genetic influences (h^2 = 57%), and the remaining variance was explained by unique environmental factors. These results are in line with the majority of prior twin studies of late-life depressive symptoms, which generally reported significant heritability (estimates ranged from 7% to 55%) and moderate to substantial unique environmental influences, with shared environmental influences playing minimal role in late-life depressive symptoms (Carmelli et al., 2000; Gatz et al., 1992; Jansson et al., 2004; Johnson et al., 2002; McGue & Christensen, 1997). The findings in Chapter 4 provide further evidence that there is moderate genetic predisposition to LLD.

LLD often co-occurs with a range of medical conditions and neuropsychiatric disorders, but the sources of covariation between these co-occurring phenotypes have not previously been explored in the literature. In Chapter 4, the genetic and environmental covariations between LLD, anxiety and hypertension were investigated, and significant genetic correlations were observed between LLD and anxiety (r_G = 0.58), and between anxiety and hypertension (r_G = 0.15). The finding that LLD and anxiety have shared genetic influences is perhaps anticipated given their considerable symptom overlap. Despite the wealth of evidence for a strong, reciprocal relationship between LLD and vascular disease, no evidence was found for a shared genetic basis between the two, most likely due to the relatively small sample size. However, only hypertension was examined as a proxy for vascular disease; genetic correlations between LLD and other vascular phenotypes, particularly cerebrovascular diseases such as stroke or cerebral microvascular dysfunction, remain to be explored.

8.2.2 Susceptibility loci for late-life depression

Chapter 5 addressed the second research aim in a systematic review of the literature of genetic association studies of LLD to identify susceptibility polymorphisms. The systematic review revealed that to date the genetic determinants of LLD have been insufficiently investigated; although polymorphisms in 23 genes have been examined in the 46 included candidate genes studies, most findings have not been replicated. Only four polymorphisms have been investigated in three or more independent samples (*APOE* ε2/ε3/ε4, *BDNF* Val66Met, *MTHFR* C677T and *SLC6A4* 5-HTTLPR), three of which were found to be significantly associated with LLD when effect sizes were pooled in the meta-analysis (*APOE* ε2/ε3/ε4, *BDNF* Val66Met and *SLC6A4* 5-HTTLPR), and the pooled effect sizes were mostly modest. However, the effect sizes may be inflated as they are greater than what is generally expected under the common disease/common variant hypothesis and may represent false positives. The review also identified a genome-wide association study of depressive symptoms that used predominantly ageing cohorts, which found a significant hit (rs40465) in a gene desert in the 5q21 region (Hek et al., 2013), but this finding is yet to be replicated. Overall, the results of the systematic review and meta-analysis in Chapter 5 indicate that no robust genetic associations with LLD have been identified to date.

The second half of Chapter 5 reported replication attempts of significant genetic associations identified in meta-analyses using a subsample from the Sydney MAS. The *APOE* ε2/ε3/ε4, *BDNF* Val66Met, *GNB3* C825T, *MTHFR* C677T and rs40465 (5q21) polymorphisms were tested for potential association with LLD; none of them significantly predicted LLD. However, that lack of significant results is not surprising due to the relatively small sample size used in this study. The inconsistent findings between genetic association studies of LLD are likely due to inadequate statistical power of prior studies, therefore it is premature to confirm or rule out any of the examined genetic polymorphisms' involvement in the pathogenesis of LLD.

8.2.3 Enduring effects of early-life trauma

Aside from genetic influences, Chapter 4 showed that the remaining variance in LLD is attributed to unique environmental influences. Many environmental risk factors for

neuropsychiatric disorders have been identified, including stressful life events such as family conflict or death of a family member. Current research suggests that adversity occurring in early life is particularly relevant to the development of neuropsychiatric disorders, since such events may coincide with critical stages of brain development and leave lasting structural and functional changes in the brain. Chapter 6 addressed the third research aim and investigated the effects of a specific type of environmental influence – early-life trauma – on LLD in the Sydney MAS cohort.

The effects of five types of childhood maltreatment, i.e. emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect, on LLD were examined, and only emotional abuse significantly predicted LLD (B = 1.397, Wald = 11.224, $p = 0.001$). In addition, the effect of exposure to Holocaust trauma when growing up on LLD was also evaluated, and it significantly predicted LLD (B = 0.807, Wald = 5.123, $p = 0.024$). These findings contribute to the limited literature on the role of early-life adversity in LLD.

Prior work investigating the relationship between childhood maltreatment and LLD is scarce, with only two studies to date reporting similar results with regard to specific types of childhood adversity that are associated with LLD. Comijs et al. (2013) reported that all types of childhood maltreatment assessed (emotional neglect, psychological abuse, physical abuse, and sexual abuse) predicted LLD, and Ege et al. (2015) found that *repeated* exposure to all types of adverse childhood experiences surveyed (parents being physically abusive to each other, being physically harmed by a parent, being sworn at by the parent, being touched sexually by an adult, being forced to sexually touch an adult, and being forced into a sexual encounter during childhood) were significant predictors of LLD. While the analyses in Chapter 6 did not fully replicate earlier findings as the other types of childhood treatment examined were not significantly associated with LLD, it provides further evidence that exposure to certain traumatic events early in life can affect distal mental health outcomes.

Potential gene-environment (GxE) interactions of childhood emotional abuse or Holocaust trauma with previously identified risk polymorphisms of depression or LLD were investigated in

the second half of Chapter 6. None of the GxE interactions tested reached statistical significance, most likely again due to the small sample size.

8.2.4 DNA methylation signatures in late-life depression

LLD is believed to involve a complex interplay of biological and environmental influences, and one mechanism by which environmental influences may exert their effects on brain development and function is through epigenetic processes, including DNA methylation. There is growing evidence to suggest DNA methylation plays an important role in depression (see Januar et al., 2015b for a review). The final research aim of examining the role of DNA methylation in LLD is addressed in Chapter 7.

Chapter 7 presents the first epigenome-wide association study (EWAS) for LLD using blood DNA methylation from a sample of older MZ twins. While the model using 17 discordant MZ twin pairs was inadequately powered and did not yield significant results, the model using the full sample of MZ twin pairs identified 69 differentially methylated probes associated with LLD. The only published DNA methylation studies of LLD to date have both focused on methylation in the promoter region of *BDNF* (Januar et al., 2015a; Kang et al., 2015); however, none of CpG probes located in or near *BDNF* were statistically significant. The pathway analysis did not identify any significantly enriched KEGG pathways after correction for multiple testing, but a number of gene ontology categories remained statistically significant after correction for multiple testing, most of which concern developmental processes. This study adds to the currently limited body of literature that shows epigenetic modifications are important in the pathophysiology of LLD.

8.3 The role of neurodevelopment in late-life depression

Taken together, the findings in this thesis highlights the potential role of neurodevelopment in the pathophysiology of LLD. Neurodevelopment is a dynamic and multifaceted process that begins in the early prenatal period and continues into adulthood, with grey matter changes following an inverted-U shape trajectory from infancy to early adulthood, and white matter increasing progressively through adolescence (Giedd & Rapoport, 2010). There are also marked

differences in gene expression, neurogenesis, and organisation of neural circuits observed at different stages across the lifespan (Salum et al., 2010).

Neurodevelopment follows regionally heterochronous maturational trajectories, with the lower-order sensorimotor cortex maturing before the higher-order association cortices (Sowell et al., 2003). As the developing brain matures, there are certain periods of heightened neuroplasticity when the brain is particularly receptive and vulnerable to insults. Exposure to stressors or insults within these windows of vulnerability or 'sensitive periods' can have profound effects on the maturation of specific cell populations, structures or cortical circuitry, resulting in long-lasting functional changes. It has been argued that the developmental timing of exposure to stressors or insults can lead to differential neurobiological phenotypes associated with differential depression risk (Andersen & Teicher, 2008; Heim & Binder, 2012; Lupien et al., 2009). For instance, genetic variation or exposure to environmental insults that influence the maturation of brain regions or circuits implicated in affective regulation (e.g., hippocampus) increase the risk for depressive disorders (Ansorge et al., 2007).

The early-life stress literature provides some evidence for sensitive periods, with stressful events experienced at different ages linked to different structural brain changes and neuropsychiatric phenotypes. Two rat studies (Andersen & Teicher, 2004; Leussis et al., 2008) and a human study (Andersen et al., 2008) showed that earlier stress exposure was associated with a smaller hippocampus while later stress exposure was associated with a smaller prefrontal cortex. It has also been shown that trauma experienced in early childhood was more strongly associated with depressive symptoms, whereas trauma experienced in late childhood or adolescence more strongly associated with post-traumatic stress symptoms in young adults (Andersen et al., 2008; Maercker et al., 2004; Schoedl et al., 2010). It has been suggested that these differences reflect the effects of hormonal changes on neuroplasticity and hypothalamic-pituitary-adrenal (HPA) axis function during puberty.

While there is currently limited research that specifically links disruptions in brain maturation to mental health outcomes in late life, the evidence from studies of adolescent and adult depression suggests neurobiological and functional abnormalities arising from these disruptions contribute

to an individual's susceptibility to depression, and the manifestation of depressive symptoms may occur later in life when neural circuits can no longer offset these dysfunctions (Ansorge et al., 2007; Lima-Ojeda et al., 2017; Salum et al., 2010). A structural equation modelling study that examined longitudinal pathways to depressive symptoms demonstrated that birth weight and economic deprivation in early childhood were associated with delayed neurodevelopment (operationalised as ages of attainment of developmental milestones), which in turn was associated with depressive symptoms in adolescence that mediated the risk of depressive symptoms in mid-adulthood. Acute stressful events occurring in adulthood, however, showed direct effects on depressive symptoms in mid-adulthood (Colman et al., 2014). A relevant concept to consider is 'brain reserve' (Klempin & Kempermann, 2007; Stern, 2009). Aberrant neurodevelopment earlier in life may result in reduced baseline neuroplasticity, rendering neural circuits less able to adapt to or compensate for the effects of various stressors in late life (e.g. age- and disease-related neurobiological changes), and consequently an underlying predisposition may be unmasked (Salum et al., 2010).

Current research suggests epigenetic factors contribute to structural and functional changes in the developing brain (Fagiolini et al., 2009), and that exposure to early-life stress can alter gene expression through epigenetic modifications and give rise to affective disorders later in life (Murgatroyd & Spengler, 2011). While the findings in Chapter 6 and Chapter 7 provide evidence for the roles of early-life stress and epigenetic changes in LLD, further research is required to elucidate whether these associations are mediated by alterations to neurodevelopmental trajectories. A better understanding of the developmental context of LLD may help develop disease-modifying interventions matched to specific neurodevelopmental stages in at-risk individuals, and may prevent or alleviate depressive symptoms later in life.

8.4 Clinical implications

Given one in every five cases of clinically relevant LLD is a new case (Smit et al., 2006), and only a third of those treated with second-generation antidepressants achieve remission (Nelson et al., 2008), prevention is key to reducing the disease burden associated with LLD.

8.4.1 Preventive strategies for late-life depression

Primary prevention strategies can be classified as universal, selective or indicated (Mrazek & Haggerty, 1994). Universal prevention addresses risk factors at the population level. Selective prevention targets subgroups of the population who are at risk of developing the disorder. Indicated prevention targets those who already manifest early or subsyndromal signs and symptoms of the disorder. While the idea of a universal prevention approach may seem appealing, a meta-analysis of prevention studies of depression showed that universal prevention was significantly less effective than selective and indicated prevention (Cuijpers et al., 2008). Moreover, universal prevention in a low-incidence disorder like depression also faces the obvious limitation of low statistical power (Okereke, 2015). There is currently no evidence-based universal preventive strategy for depression (Schoevers et al., 2006). In meta-analyses of prevention studies using psychological interventions, selective and indicated prevention approaches were found to significantly reduce the incidence of depressive disorders by 19% to 28% compared to the treatment-as-usual control group, though the number of people who would need to receive preventive intervention in order to prevent one new case of LLD was high (number needed to treat = 20-22) (Cuijpers et al., 2008; van Zoonen et al., 2014),

Randomised controlled trials of selective prevention of LLD have been conducted typically in older adults with medical conditions such as cardiovascular disease (Andreeva et al., 2012; Giltay et al., 2011; Salminen et al., 2005) or stroke (Almeida et al., 2010; Robinson et al., 2008), or in older adults in residential aged care (Dechamps et al., 2010; Haight et al., 1998; Konnert et al., 2009). Different preventive interventions including antidepressants, psychosocial interventions, lifestyle interventions and nutritional supplementation were used in different studies, with mixed results reported. It has been suggested that the inconsistent findings can partly be explained by the fact that the measures used to identify at-risk groups in these studies are not those found to be most strongly associated to LLD risk (Okereke, 2015).

Schoevers et al. (2006) compared selective and indicated prevention models using data from the Longitudinal Aging Study Amsterdam and found that indicated prevention offers greater cost-effectiveness than selective prevention. Efficiency can also be further improved by taking

additional risk indicators into account, including female sex, low education, presence of chronic diseases, functional impairment, an above-average number of depressive symptoms, and a small social network (Smit et al., 2006). Similarly, Lyness et al. (2009) found that a combination of risk indicators, including minor or subsyndromal depression, functional impairment, and a history of major or minor depression, can identify a group of older adults at high risk for incident depression, in which successful treatment of five individuals would prevent one case of incident depression.

A few randomised controlled trials for indicated prevention of LLD have been conducted. Ciechanowski et al. (2004) explored the effects of a home-based program that comprised problem-solving treatment, social and physical activation, and potential recommendations to primary care physicians regarding antidepressants in a sample of older adults with minor depression or dysthymia. Older adults assigned to the intervention group were more likely to have a significant reduction in depressive symptoms, to achieve complete remission compared to those receiving usual care. Three other indicated prevention studies were based on the stepped-care model, but the results are mixed. In two studies conducted by van't Veer-Tazelaar and colleagues (2009; 2011), older adults presenting with subthreshold depression and anxiety symptoms in primary care were randomised either to a stepped-care program that involved watchful waiting, cognitive behavioural therapy-based bibliotherapy, cognitive behavioural therapy-based problem-solving treatment, and referral to primary care for medication if required, or usual care. The 12- and 24-month incidence was halved by the intervention, when compared to usual care. Dozeman et al. (2012) used a similar stepped-care program comprising watchful waiting, activity-scheduling, life review and consultation with general practitioner, and visit to general practitioner for additional treatment if required to prevent depression and anxiety disorders in older adults in residential aged care. While the program did not reduce the overall incidence of depression and anxiety disorders combined, it successfully reduced the incidence of depression. A stepped-care program with components of watchful waiting, bibliotherapy, individual cognitive behavioural therapy, and indicated treatment designed to prevent the relapse of depression in older adults showed no difference in outcomes compared to usual care (Apil et al., 2012). In fact, the 12-month follow-up even showed that older adults in

the stepped-care program required significantly more new treatment than those who received usual care (Apil et al., 2014).

Recently, researchers have also explored the role of lifestyle factors in preventing LLD. For example, there is evidence that adherence to a Mediterranean-style diet is associated with a lower number of incident depressive symptoms among community-dwelling older adults (Skarupski et al., 2013) and a lower risk of incident clinical depression in a subgroup of community-dwelling older adults with type 2 diabetes (Sánchez-Villegas et al., 2013). Other studies have examined the potential benefits of physical activity in preventing LLD, with a number of studies reporting protective effects of physical activity in preventing incident depression or depressive symptoms in older adults across long follow-up periods (Bots et al., 2008; Hamer et al., 2009; Ku et al., 2009; Lucas et al., 2011; Strawbridge et al., 2002), though one study only found a cross-sectional association (Kritz-Silverstein et al., 2001). Exercise using video games also appeared to improve depressive symptoms in older adults with subsyndromal depression (Rosenberg et al., 2010).

In order for prevention efforts to be efficient, it is important to identify older adults who are most likely to benefit from such programs. However, the specificity of most risk indicators for depression is low. It has been suggested that advances in biomarker research may improve the identification of at-risk older adults as well as discover new possibilities for prevention, so that the most appropriate preventive strategies can be directed to those who need them most (Cuijpers et al., 2015; Reynolds et al., 2012). For example, meta-analyses have shown differential associations between *BDNF* Val66Met genotypes and antidepressant response (Zou et al., 2010), and between *SLC6A4* 5-HTTLPR genotypes and antidepressant efficacy (Porcelli et al., 2012) in major depressive disorder (MDD), which may reflect differences in underlying neurobiological disruptions. By the same token, it is anticipated that older adults of different genotypes may not respond equally to the same preventive strategies. In assessing which biomarkers will be most useful in identifying the optimal target group for prevention, decision tree models can be used to evaluate the joint predictive power of various combinations of risk indicators (Batterham et al., 2009; Smits et al., 2008; Wong et al., 2012). Decision tree models may also identify potentially modifiable risk factors that contribute significantly to the risk of incident depression, so

prevention or early intervention programs can be tailored to target these risk factors (Batterham et al., 2009).

Given the effect sizes of the risk polymorphisms identified in Chapter 5 and the DNA methylation changes found in Chapter 7 are generally modest and replication is required, more research is needed to identify robust genetic and epigenetic markers that can be used as risk indicators in identifying older adults at risk of developing LLD. On the other hand, screening for anxiety symptoms or a history of early-life trauma in older adults, particularly individuals already presenting with subsyndromal depressive symptoms, may be useful in identifying those at high risk of developing clinical depression.

8.4.2 Ethical challenges of predictive genetic or epigenetic testing

While it has been argued that the incorporation of biomarkers may help identify optimal target groups for the prevention of LLD, predictive testing for neuropsychiatric disorders provokes certain ethical questions.

Due to the multifactorial nature of neuropsychiatric disorders, most implicated single-nucleotide polymorphisms contribute very modest effects to susceptibility of the disorder, and are of no clinical value when considered in isolation. Moreover, susceptibility genes are neither necessary nor sufficient to cause the disorder, and merely contribute to the *probability* of developing the disorder. This probability is also influenced by GxE interactions through epigenetic mechanisms as well as possible GxE correlations (Kendler & Baker, 2007). Therefore, predictive genetic testing for individual susceptibility genes currently has limited usefulness in the context of neuropsychiatric disorders and is discouraged until the information can be put to effective preventive or therapeutic use (Nuffield Council on Bioethics, 1998). In view of the growing consensus that many genetic loci of small effects likely explain the genetic liability of depression, several studies have examined polygenic risk profiles for depression. These studies found that polygenic risk scores significantly predicted depression, but the variance explained was diminutive (up to 1%) (Bigdeli et al., 2017; Demirkan et al., 2011; Musliner et al., 2014). Further identification of risk loci of LLD in large-scale genome-wide association studies (GWAS) will allow for better prediction, and then predictive genetic testing may be justified.

Stigmatisation of neuropsychiatric disorders is still common nowadays, and it is unclear whether an increased knowledge of genetic influences of these disorders would necessarily help reduce the stigma as such genetic information is probabilistic and could be interpreted in different ways (Singh & Rose, 2009). Moreover, genetic information associated with neuropsychiatric disorders could potentially become a basis for discrimination in employment and insurance (Lakhan et al., 2010; Nuffield Council on Bioethics, 1998).

While there is relatively less published research on the ethical issues surrounding the use of other types of biomarkers in neuropsychiatric disorders, the points discussed above also apply to DNA methylation changes in that their effects are also small and are of low specificity. It is possible that future advances in genetic and biomarker research of neuropsychiatric disorders would offer increased precision in identifying at-risk older adults, but robust and replicable susceptibility polymorphisms or biomarkers need to be identified before testing can be justified.

8.4.3 Therapeutic options for late-life depression

Research shows that all antidepressants that are currently clinically available increase adult hippocampal neurogenesis, though neurogenesis is not always necessary for the behavioural effects of antidepressants (Anacker & Hen, 2017; Eliwa et al., 2017). However, impaired neurogenesis and dentate gyrus dysfunction may be used as biomarkers to guide treatment selection (Anacker & Hen, 2017). Currently, there is evidence that magnetic resonance spectroscopy can be used to detect and quantify neural stem and progenitor cells *in vivo* in the human brain (Manganas et al., 2007). Recent advances in [^{18}F]fluoro-L-thymidine positron emission tomography may also prove useful for quantifying and detecting changes in neurogenesis in humans in the future (Rueger et al., 2010; Tamura & Kataoka, 2017; Tamura et al., 2016). In older adults who show significant neurogenesis deficits or dentate gyrus functional impairments, treatment strategies that have been shown to be effective in increasing neurogenesis should be used, for example exercise (Fabel & Kempermann, 2008; Ryan & Nolan, 2016) or environmental enrichment (Olson et al., 2006).

On the other hand, there is preliminary evidence that epigenetic mechanisms moderate or mediate antidepressant effects, with lower DNA methylation in a number of CPG sites within the

BDNF promoter observed in depressed adults who responded to treatment (Tadić et al., 2013), and increased *BDNF* expression following citalopram treatment (Lopez et al., 2012). EWAS hold promise for the identification of mechanisms and pathways involved in depression, which will allow novel treatments to be developed. As more risk loci are identified in EWAS, epigenomic profiling may also be used for depression subtyping and to guide treatment selection (Menke & Binder, 2014).

It was discussed in Chapter 1 that LLD represents a significant public health challenge due to its association with increased disability, morbidity and mortality, which results in substantially higher health care costs. LLD is also closely related to dementia (Diniz et al., 2013a; Singh-Manoux et al., 2017), which is ever increasing in prevalence in an age of global population ageing. More accurate identification of older adults at high risk of developing depression or already presenting with subthreshold depressive symptoms, as well as the development of more targeted preventive and treatment strategies is anticipated to effectively reduce the disease burden associated with these late-life disorders.

8.5 Methodological and conceptual considerations and limitations

Despite tremendous advances in genetic, neuroscience and neuroimaging techniques in the past few decades that have enhanced our understanding of brain function, these insights have not translated into tools that can be used in psychiatric clinical practice. Specific methodological limitations pertaining to each study have been discussed within their respective chapters, but a number of broader methodological and conceptual issues relevant to neuropsychiatric research, and how these issues contribute to the gap between bench and bedside are discussed below.

8.5.1 Small sample sizes

As was demonstrated in the studies presented in this thesis, inadequate statistical power is a common problem in genetic/genomic and epigenetic/epigenomic studies of neuropsychiatric disorders. It is believed that common variants typically confer very small effects (*OR*s < 1.1) in a complex disorder, and are unlikely to be detected with the sample sizes of existing individual

studies (O'Donovan, 2015). This highlights that large-scale, multi-site studies, and especially international collaborative efforts are vital in the quest for genetic and epigenetic underpinnings of LLD. A number of existing international research consortia investigating neuropsychiatric or neurodegenerative disorders have shown that meta-analyses of data from multiple large population-based cohorts can yield a treasure trove of new knowledge of disease-relevant markers, these consortia include the Psychiatric Genomics Consortium (PGC) (O'Donovan, 2015; Sullivan, 2010), the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) (Psaty et al., 2009), and the Enhancing Neuroimaging Genetics Through Meta-Analysis (ENIGMA) Network (Thompson et al., 2014). It is expected that leveraging data from these established cohorts will also allow rare or low-frequency variants as well as epigenetic modifications to be comprehensively examined in the future.

8.5.2 Definition of depression

Depression caseness was defined as a GDS-15 score of six or above or self-reported antidepressant use across all studies in this thesis. The rationale for employing this composite outcome variable was to avoid overlooking participants who were depressed but reported lower GDS-15 scores due to treatment with antidepressants, and a similar method to create a composite based on Centre for Epidemiological Studies – Depression (CES-D) scale scores and self-reported antidepressant use has been employed in an earlier twin study of late-life depressive symptoms (Jansson et al., 2004). Antidepressants may be prescribed for various medical conditions other than depression, such as insomnia and anxiety disorders (Wong et al., 2017), which could have led to an under- or overestimation of effect sizes reported in this thesis due to misclassification.

8.5.3 Heterogeneity in depression

It is well recognised that depression is a highly heterogeneous disorder. A study examining depression symptom profiles in a sample of 3,703 depressed outpatients found over 1,000 unique combinations of Diagnostic and Statistical Manual of Mental Disorders (DSM-5) depression symptoms (Fried & Nesse, 2015), suggesting that individuals with the same diagnostic label can have vastly different clinical presentations. In addition, how depression is measured also

contributes to the heterogeneity. For instance, a survey in 2006 identified 280 measures of depressive severity that have been developed and published since 1918, with the different scales showing a marked degree of variability (Santor et al., 2006). Depression studies generally include only one particular scale as the outcome measure, implicitly assuming that various measures of depression assess the same latent construct, and therefore can be used interchangeably and study findings are generalisable. However, recent studies showed that depression rating scales are often multifactorial and do not assess a single underlying construct (Fried et al., 2016), and there is limited content overlap between different depression rating scales (Fried, 2017). All empirical studies in this thesis used the GDS-15 to assess depressive symptoms, which does not contain somatic symptoms as they may be secondary to age-related changes or physical conditions that are common in late life. It also offers ease of administration in an epidemiological study with its yes/no format. This is supplemented by self-reported antidepressant use information to also include older adults who report subthreshold depressive symptoms following successful treatment. This broad definition of depression is expected to capture the majority of older adults who display clinically relevant depressive symptoms within the cohorts.

The marked heterogeneity in depression unquestionably deserves more attention as there is growing evidence that such heterogeneity is clinically meaningful, and may be reflective of different neurobiological pathways involved as well as different trajectories of the disorder. Studies of both adult and late-life depression have shown that differential associations between depression symptom profiles and biological correlates or outcomes. For instance, a number of studies have compared biological alterations in melancholic and atypical depression, and found that the atypical subtype is more strongly associated with metabolic and immuno-inflammatory dysregulation (Penninx et al., 2013). Another example is a study that examined treatment outcome patterns associated with symptom combinations in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, which found a small but significant effect that citalopram treatment outcomes vary as a function of depression symptom combinations (Olbert et al., 2016). In the case of LLD, features such as concomitant delusions, sleep disturbance or executive dysfunction also predicted poorer outcomes (Baldwin, 1988; Naismith et al., 2011; Pimontel et al., 2016). These findings indicate that the heterogeneity observed in depressed older

adults may reflect different aetiologies, with subtypes associated with different disease course and prognosis, therefore the nature of the biological dysregulation involved may carry prognostic or treatment implications.

8.5.4 Polygenic and multifactorial nature of late-life depression

The polygenic and multifactorial nature of LLD is another reason why genetic association studies have often failed to detect replicable susceptibility genes. It is believed that the genetic variation in depression is made up of joint effects of multiple common loci of small effects (Flint & Kendler, 2014), some of which may only be unmasked when gene-gene or GxE interactions are considered (Lesch, 2004; Uher, 2008). Since genetic and environmental factors do not act in isolation, research focusing on simple associations between a certain risk and the disease phenotype can only produce limited insights into aetiology.

Arguably, convergence from multiple lines of evidence from different 'omics' studies, combined using systems biology or network models, may help uncover the underlying neurobiological pathways and networks in LLD (Alawieh et al., 2012; Fernandes et al., 2017; Silbersweig & Loscalzo, 2017).

8.5.5 Inherent imprecision in a descriptive nosology

At present, psychiatric disorders are diagnosed using criteria based on phenomenology rather than aetiology or pathophysiology, which results in diagnostic categories of questionable biological validity as well as substantial within-disorder heterogeneity and between-disorder overlap (Kapur et al., 2012; Wardenaar & de Jonge, 2013). This largely reflects the poor knowledge we have on the pathophysiology underlying these disorders, but in recent years a growing chorus of voices has argued that the inherent imprecision in the descriptive and categorical nosology of psychiatry is impeding progress in aetiological research and the identification of new treatments (Cuthbert & Insel, 2013; Demkow & Wolańczyk, 2017; Hyman, 2010; Kapur et al., 2012; Lozupone et al., 2017; Scarr et al., 2015).

A growing body of research shows that patterns of genetic liability and neurobiological abnormalities cut across conventional diagnostic boundaries, challenging the view that

psychiatric disorders are discrete entities. For example, genome-wide association studies have identified specific single-nucleotide polymorphisms that show cross-disorder associations as well as significant genetic correlations between the disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013a, 2013b; International Schizophrenia Consortium, 2009). Meta-analyses of peripheral biomarkers of major psychiatric disorders have concluded that significant variations of five commonly studied biomarkers, i.e. brain-derived neurotrophic factor (BDNF), tumour necrosis factor (TNF)-α, interleukin (IL)-6, C-reactive protein (CRP) and cortisol, occur in at least some state of schizophrenia, depression or bipolar disorder (Pinto et al., 2017). Furthermore, factor analyses have shown that psychiatric symptoms or disorders can be explained by a common general psychopathology factor (Caspi et al., 2013; Stochl et al., 2014). A population-based family study also showed that the individuals with a parental history of psychiatric disorders are at an increased risk of developing not only concordant disorders, but also a wide range of other psychiatric disorders (Dean et al., 2010). Taken together, these findings suggest that there are common underlying vulnerabilities to psychiatric disorders, which may place individuals on different trajectories following different environmental exposures. They also help explicate the high degree of heterogeneity among individuals who received the same diagnostic label.

With the imprecise and non-biologically based categories in our current diagnostic systems, it cannot be expected that any neurobiological abnormality will show a strong one-to-one mapping with a DSM- or International Classification of Diseases (ICD)-defined psychiatric disorder. It is for this reason that these diagnostic categories are perhaps not ideal to be used as a benchmark for the discovery of biomarkers. Since our current understanding of the biological underpinnings of psychiatric disorders is still limited, and recent genetic and neurobiological findings have not been adequately replicated or validated, it would undoubtedly be premature to have a complete diagnostic reclassification. However, alternative approaches are necessary to move beyond the reification of disorders in order to ultimately arrive at an aetiology-based classification system.

Stratified psychiatry is an approach that has been put forward as a promising alternative. Instead of pursuing biological diagnostic or screening tests for conventional neuropsychiatric disorders, a better option is to identify biomarkers that 'subtype' these disorders or re-cluster symptoms for prognosis prediction and treatment selection (Hyman, 2010; Kapur et al., 2012; Scarr et al., 2015). Stratification into biologically homogenous subtypes or re-clustering of symptoms based on neurobiological evidence would likely also improve the chances of identifying the underlying neurobiological disturbances in research studies (Hyman, 2010; Stephan et al., 2016). Effective stratification is expected to involve a combination of markers, which may be biological, cognitive or psychological (Kapur et al., 2012).

8.6 Future avenues of research

The findings from the studies in this thesis and the methodological and conceptual considerations discussed above highlight several areas for further consideration.

1. The genetic correlation between LLD and hypertension was examined and was found to be non-significant. However, no other vascular phenotypes have been examined, and the role of genetic influences on the co-occurrence of LLD and other vascular phenotypes remains unclear. Future twin studies should examine a range of vascular variables, particularly cerebrovascular phenotypes that have been shown to be associated with a high risk of depression, in larger older twin samples.
2. The systematic review in Chapter 5 revealed a dearth of genetic studies of specifically addressing LLD, particularly those using cases where the onset of depression occurred later in life. Further large-scale cohort studies combined with international collaborative efforts are recommended to achieve the sample sizes required for adequate statistical power in genetic association studies, which may allow robust susceptibility genes to be eventually identified. Replication of susceptibility variants identified is also necessary.
3. The DNA methylation study in Chapter 7 presents preliminary evidence for DNA methylation changes in LLD. Further research is warranted to replicate or validate these findings before conclusions can be drawn.

4. The analyses carried out in this thesis focused on simple associations of genetic, environmental and epigenomic influences with depression. Novel approaches, such as multi-omics approaches combined with systems biology or network models, are needed to further our understanding of the neurobiological perturbations underlying LLD due to the complex nature of the disorder.
5. The clinical and biological heterogeneity in LLD deserves careful consideration, as it may account for a substantial proportion of inconsistent findings in the literature. Stratification of depressed older adults into biologically homogeneous subgroups or by biologically based symptom clusters could reduce heterogeneity and increase the likelihood of identifying biomarkers of clinical utility.
6. Further studies are required to identify older adults who would most likely benefit from prevention programs of LLD. A wide range of risk factors should be explored using methods like decision tree models that can evaluate the combined risk of various risk factors.
7. While it is evident that prevention is key to reducing the disease burden associated with LLD, the current evidence for preventive strategies is limited; more research is essential to develop effective preventive strategies for LLD.

8.7 Concluding remarks

The body of research presented in this thesis was designed to obtain new insights into the pathophysiology of LLD through examining the genetic, environmental and epigenomic influences on the disorder. This thesis provides novel contributions to furthering our understanding in the following ways:

1. This is the first study that examined the shared genetic and environmental influences on LLD and commonly co-occurring phenotypes, which found shared genetic factors underlying LLD and anxiety. This suggests older adults manifesting anxiety symptoms may be at greater risk of developing LLD, which may have implications for prevention.
2. A comprehensive systematic review of genetic association studies of LLD was conducted, and it revealed that there is a scarcity of studies and many findings have also not been

replicated. Further research is warranted to improve our understanding on the genetic determinants of LLD.

3. Through examining the effects of childhood maltreatment and war-related trauma on LLD, it was demonstrated that early-life trauma can affect distal mental health outcomes. Screening for a history of early-life trauma may be useful in identifying at-risk individuals at which preventive efforts should be targeted.
4. The first epigenome-wide association study of LLD was conducted and a number of differentially methylated probes were identified. The overrepresentation test showed that the results were enriched for many developmental processes, including central nervous system development, as well as neurogenesis.

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APPENDIX A. SUPPLEMENTAL TABLES

Table A.1. Full search strategy used in MEDLINE.

1	(depress* or mood disorder* or affective disorder* or unipolar).ti,ab.
2	Depression/ge [Genetics]
3	Depressive Disorder/ge [Genetics]
4	Depressive Disorder, Major/ge [Genetics]
5	or/1-4
6	(older or elder* or geriatric or late life or late onset).ti,ab.
7	Aged/
8	"Aged, 80 and over"/
9	or/6-8
10	(gene? or genetic* or genotyp* or polymorphism* or SNP* or allel* or variant* or chromosom*).ti,ab.
11	Genetic Predisposition to Disease/
12	Genotype/
13	Polymorphism, Genetic/
14	Polymorphism, Single Nucleotide/
15	or/10-14
16	(control* or compar* or cross sectional or genome wide association).ti,ab.
17	Case-Control studies/
18	Comparative Study/
19	Cross-Sectional Studies/
20	Genetic Association Studies/
21	Genome-Wide Association Study/
22	or/16-21
23	Humans/
24	5 and 9 and 15 and 22 and 23
25	limit 24 to (english and journal article)

Table A.2. Characteristics of studies included in APOE ε2/ε3/ε4 meta-analysis.

First author (year)	Country	Ethnicity of sample	Age *M* ± *SD* (range)	% female	Phenotype / instrument	Cases		Controls		Source of sample
						ε4-	ε4+	ε4-	ε4+	
Class (1997)	USA	African American	76.6±7.3	Not reported	GDS-30	ε4-: 7.1±5.3 (n=67); ε4+: 6.3±5.0 (n=45)				Community
Harwood (1999)	USA	Majority Caucasian	75.2±7.5	59.9	HAM-D≥12	40	21	336	109	Community
Nebes (2001)	USA	Majority Caucasian	73.6±3.4	Not reported	GDS-30	ε4-: 2.9±3.2 (n=71), ε4+: 3.6±4.0 (n=21)				Community
Butters (2003)	USA	Majority Caucasian	69.8±6.9	65.8	DSM-IV MD (SCID)	124	36	109	47	Community
Steffens (2003a)	USA	Majority Caucasian	(65-100)	52.0	DSM-IV MD	161	65	2267	999	Community
Steffens (2003b)	USA	Majority Caucasian	(≥60)	66.9	DSM-IV MD	103	42	75	25	Community
Schahab (2006)	Germany	Caucasian	70.2±8.5	54.0	DSM-IV MD	138	42	168	57	Hospital
Taylor (2007a)	USA	Majority Caucasian	70.1±7.1	67.6	DSM-IV MD	164	62	109	35	Community
Slifer (2009)	USA	Caucasian	73.7±7.2	61.8	GDS-30≥10	53	14	344	113	Community
Sohrabi (2009)	Australia	Majority Caucasian	67.3±6.6	64.5	GDS-30	ε4-: 3.76±3.59 (n=55), ε4+: 4.98±4.98 (n=83)				Community
Sureshkumar (2012)	India	Asian	(≥50)	45.2	HAM-D≥11	20	11	28	3	Community
Chen (2013b)	China	Asian	67.5±6.0	68.4	CCMD-3 depression & GDS-30≥11	55	9	29	1	Hospital
Nose (2013)	Japan	Asian	73.6±5.6	57.6	GDS-15≥6	129	30	460	119	Community
						ε2	ε3/ε4	ε2	ε3/ε4	

First author (year)	Country	Ethnicity of sample	Age *M* ± *SD* (range)	% female	Phenotype / instrument	Cases			Controls			Source of sample
						ε4-		ε4+	ε4-		ε4+	
Schmand (1998)	Netherlands	Majority Caucasian	67.3±5.8	54.0	GMS-AGECAT depression≥3	6	43	22	14	104	35	Community
Papassotiropoulos (1999)	Germany	Caucasian	69.4±10.0	58.1	Clinical diagnosis	25	88	47	33	109	49	Community
Hwang (2006b)	Taiwan	Asian	74.4±7.3	42.3	DSM-IV MD	11	80	20	17	105	22	Hospital
Traykov (2007)	France	Caucasian	75.3±7.2	66.3	DSM-IV MD	2	8	14	15	77	30	Hospital

Abbreviations: AGECAT – Automated Geriatric Examination for Computer Assisted Taxonomy; CCMD-3 – Chinese Classification of Mental Disorders, 3rd edition; DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, 4th edition; GDS – Geriatric Depression Scale; GMS – Geriatric Mental State Schedule; HAM-D – Hamilton Rating Scale for Depression; MD – major depression; SCID – Structured Clinical Interview for DSM-IV Axis I Disorders

Table A.3. Characteristics of studies included in BDNF Val66Met meta-analysis.

First author (year)	Country	Ethnicity of sample	Age *M* ± *SD*	% female	Phenotype/ instrument	Genotypes					Alleles				Source of sample
						Cases			Controls				Cases		Controls	
						Met/Met	Val/Met	Val/Val	Met/Met	Val/Met	Val/Val	Met	Val	Met	Val	
Taylor (2007b)	USA	Caucasian	69.7±7.1	65.8	DDES	10	85	150	1	22	71	105	385	24	164	Community
Lin (2009)	Taiwan	Asian	78.4±5.3	24.0	DSM-IV MD	45	63	47	31	98	66	153	157	160	230	Hospital
You (2010)	China	Asian	72.5±5.3	54.3	DSM-IV MD & HAM-D≥17	39	68	37	25	58	27	146	142	108	112	Hospital
Kanellopoulos (2011)	USA	Caucasian	71.6±6.4	62.5	DSM-IV-TR MD (SCID) & HAM-D≥18	3	13	17	0	14	12	19	47	14	38	Community

Abbreviations: DDES – Duke Depression Evaluation Schedule; DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, 4th edition; HAM-D – Hamilton Rating Scale for Depression; MD – major depression; SCID – Structured Clinical Interview for DSM-IV Axis I Disorders

Table A.4. Characteristics of studies included in MTHFR C677T meta-analysis.

First author (year)	Country	Ethnicity of sample	Age *M* ± *SD*	% female	Phenotype / instrument	Genotypes						Alleles				Source of sample
						Cases			Controls			Cases		Controls		
						T/T	C/T	C/C	T/T	C/T	C/C	T	C	T	C	
Almeida (2005)	Australia	Majority Caucasian	74.7±4.4	100.0	BDI	3	26	13	26	87	85	32	52	139	257	Community
Chen (2005)	Taiwan	Asian	72.3±5.4	67.8	DSM-IV MD & HAM-D≥15	2	15	22	0	9	11	19	59	9	31	Hospital
Pan (2009)	USA	Caucasian	69.5±6.9	68.4	DDES	19	79	72	9	44	30	117	223	62	104	Community
El-Batch (2010)	Egypt	Egyptian	61.2±5.4	67.3	DSM-IV-TR MD	10	14	8	1	5	14	34	30	7	33	Hospital
Lan (2012)	Taiwan	Asian	80.6±5.3	0.0	GDS-SF	T/T: 3.1±2.8 (n=64), C/T: 3.2±2.8 (n=141), C/C: 3.7±3.2 (n=118)										Community

Abbreviations: BDI – Beck Depression Inventory; DDES – Duke Depression Evaluation Schedule; DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, 4th edition; GDS-SF – Geriatric Depression Scale-short form; HAM-D – Hamilton Rating Scale for Depression; MD – major depression

Table A.5. Characteristics of studies included in SLC6A4 5-HTTLPR meta-analysis.

First author (year)	Country	Ethnicity of sample	Age *M* ± *SD*	% female	Phenotype / instrument	Genotypes						Alleles				Source of sample
						Cases			Controls			Cases		Controls		
						S/S	S/L	L/L	S/S	S/L	L/L	S	L	S	L	
Grünblatt (2006a)	Austria	Caucasian	75.8±0.5	56.4	DSM-IV major/ minor/ subsyndromal depression (SCID)	27	49	34	52	164	144	103	117	168	452	Community
Taylor (2007a)	USA	Majority Caucasian	70.1±7.1	67.6	DSM-IV MD	42	102	82	21	70	53	186	266	112	176	Community
Alexopoulos (2009)	USA	Caucasian	71.1±6.6	63.0	DSM-IV MD (SCID) & HAM-D≥18	1	17	9	0	21	6	19	35	21	33	Community
Goldman (2010)	Taiwan	Asian	65.9±9.9	46.2	CES-D	S/S: 4.7±5.3 (n=449), S/L: 4.9±5.7 (n=367), S/XL: 5.6±6.0 (n=61), L/L: 4.1±5.7 (n=83), L/XL: 2.9±3.5 (n=24)										Community
Mendes (2013)	Brazil	Majority non-Caucasian	70.5±7.4	48.5	GDS-15≥6 & clinical diagnosis	15	17	11	28	73	56	47	39	129	185	Community

Abbreviations: CES-D – Centre for Epidemiologic Studies Depression Scale; DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, 4th edition; HAM-D – Hamilton Rating Scale for Depression; GDS – Geriatric Depression Scale; MD – major depression; SCID – Structured Clinical Interview for DSM-IV Axis I Disorders

APPENDIX B. ADDITIONAL ANALYSES FOR CHAPTERS 5 AND 6

Sensitivity analyses for the candidate gene logistic regressions in Chapter 5 were performed with depression defined as a GDS-15 score of 12 or above or self-reported antidepressant use (B.1). Alternative analyses using multiple regression with continuous GDS-15 scores as the dependent variable were also carried out to test the candidate gene associations in Chapter 5 (B.2), the early-life trauma effects (B.3) and the gene-environment interactions in Chapter 6 (B.4). The results are presented below. All but one of the associations tested were non-significant, confirming the results of the dichotomous analyses.

B.1 Sensitivity analyses for associations of candidate polymorphisms and LLD (depression caseness defined as GDS-15 score of 12 or above or self-reported antidepressant use)

Table B.1. Logistic regression of *APOE* ε2 carrier status in predicting LLD.

Predictor	*B*	*SE*	*p*	OR
APOE ε2	-0.25	0.36	0.48	0.78 (0.39-1.57)
Age (mean-centred)	0.00	0.03	0.97	1.00 (0.95-1.05)
Sex	0.29	0.25	0.25	1.33 (0.82-2.18)
(Constant)	-2.38	0.20	<0.001	0.09
Model χ^2 = 1.80, *df* = 3, *p* = 0.62				
-2LL = 493.43, Nagelkerke R^2 = 0.005				
Hosmer-Lemeshow test *p* = 0.10				

Table B.2. Logistic regression of *APOE* ε4 carrier status in predicting LLD.

Predictor	*B*	*SE*	*p*	OR
APOE ε4	0.14	0.28	0.63	1.15 (0.66-1.99)
Age (mean-centred)	0.00	0.03	0.98	1.00 (0.95-1.05)
Sex	0.29	0.25	0.26	1.33 (0.81-2.17)
(Constant)	-2.45	0.21	<0.001	0.09
Model χ^2 = 1.51, *df* = 3, *p* = 0.68				
-2LL = 493.71, Nagelkerke R^2 = 0.004				
Hosmer-Lemeshow test *p* = 0.08				

Table B.3. Logistic regression of *BDNF* Val66Met genotype in predicting LLD.

Predictor	*B*	*SE*	*p*	OR
BDNF Val66Met				
Val/Met	0.10	0.26	0.71	1.10 (0.66-1.83)
Met/Met	-0.35	0.75	0.64	0.70 (0.16-3.05)
Age (mean-centred)	0.00	0.03	0.98	1.00 (0.95-1.05)
Sex	0.27	0.25	0.28	1.32 (0.80-2.15)
(Constant)	-2.43	0.22	<0.001	0.09
Model χ^2 = 1.71, *df* = 4, *p* = 0.79				
-2LL = 493.51, Nagelkerke R^2 = 0.005				
Hosmer-Lemeshow test *p* = 0.06				

Table B.4. Logistic regression of *GNB3* C825T genotype in predicting LLD.

Predictor	*B*	*SE*	*p*	OR
GNB3 C825T				
C/T	0.00	0.26	1.00	1.00 (0.61-1.65)
T/T	0.19	0.44	0.66	1.21 (0.51-2.86)
Age (mean-centred)	0.00	0.03	0.99	1.00 (0.95-1.05)
Sex	0.28	0.25	0.27	1.32 (0.81-2.16)
(Constant)	-2.43	0.23	<0.001	0.09
Model χ^2 = 1.48, *df* = 4, *p* = 0.83				
-2LL = 493.74, Nagelkerke R^2 = 0.004				
Hosmer-Lemeshow test *p* = 0.46				

Table B.5. Logistic regression of *MTHFR* C677T genotype in predicting LLD.

Predictor	*B*	*SE*	*p*	OR
MTHFR C677T				
C/T	0.43	0.27	0.11	1.53 (0.91-2.59)
T/T	-0.20	0.43	0.64	0.82 (0.36-1.89)
Age (mean-centred)	0.00	0.03	0.96	1.00 (0.95-1.05)
Sex	0.28	0.25	0.28	1.32 (0.80-2.16)
(Constant)	-2.61	0.27	<0.001	0.07
Model χ^2 = 5.38, *df* = 4, *p* = 0.25				
-2LL = 489.85, Nagelkerke R^2 = 0.015				
Hosmer-Lemeshow test *p* = 0.95				

Table B.6. Logistic regression of rs40465 genotype in predicting LLD.

Predictor	*B*	*SE*	*p*	OR
rs40465				
T/G	-0.05	0.25	0.83	0.95 (0.58-1.55)
G/G	-1.12	0.61	0.07	0.33 (0.10-1.09)
Age (mean-centred)	0.00	0.03	0.95	1.00 (0.95-1.05)
Sex	0.29	0.25	0.25	1.33 (0.82-2.18)
(Constant)	-2.32	0.23	<0.001	0.10
Model χ^2 = 5.80, *df* = 4, *p* = 0.21				
-2LL = 489.42, Nagelkerke R^2 = 0.016				
Hosmer-Lemeshow test *p* = 0.37				

B.2 Secondary analyses for associations of candidate polymorphisms and LLD using continuous GDS-15 score as the dependent variable

Associations between the candidate polymorphisms and LLD were investigated using separate multiple regression models, with GDS-15 score as the dependent variable. The predictors in each model included the polymorphism of interest, age and sex. The common homozygote was used as the base group in each model. In line with the results of the logistic regression analyses, none of the candidate polymorphisms were associated with the GDS-15 score. However, older age and male sex significantly predicted the GDS-15 score, which shows that subclinical depressive symptoms rather than clinically relevant depressive symptoms (as represented by a GDS-15 score of 6 or above) are more likely to be associated with age and sex.

B.2.1 APOE *ε2*

The model explained 2.3% of the variance of GDS-15 scores (R^2 = 0.023, $F(3,783) = 6.20$, p < 0.001). Age and sex significantly predicted GDS-15 scores (B = 0.05, $p < 0.001$ and b = -0.28, p = 0.04 respectively).

Table B.7. Multiple regression of *APOE* ε2 carrier status in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
APOE ε2	0.001	0.18	0.004	1.00
Age (mean-centred)	0.05	0.01	3.83	**<0.001**
Sex	-0.28	0.14	-2.08	**0.04**
(Constant)	2.62	0.22	11.73	<0.001

B.2.2 APOE *ε4*

The model explained 2.3% of the variance of GDS-15 scores ($R^2 = 0.023$, $F(3,783) = 6.20$, $p < 0.001$). Age and sex significantly predicted GDS-15 scores ($B = 0.05$, $p < 0.001$ and $B = -0.28$, $p = 0.04$ respectively).

Table B.8. Multiple regression of *APOE* ε4 carrier status in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
APOE ε4	-0.01	0.16	-0.09	0.93
Age (mean-centred)	0.05	0.01	3.82	**<0.001**
Sex	-0.28	0.14	-2.09	**0.04**
(Constant)	2.63	0.23	11.58	<0.001

B.2.3 BDNF *Val66Met*

The model explained 2.3% of the variance of GDS-15 scores ($R^2 = 0.023$, $F(3,783) = 6.20$, $p < 0.001$). Age and sex significantly predicted GDS-15 scores ($B = 0.05$, $p < 0.001$ and $B = -0.28$, $p = 0.04$ respectively).

Table B.9. Multiple regression of *BDNF* Val66Met genotype in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
BDNF Val66Met	0.02	0.12	0.12	0.90
Age (mean-centred)	0.05	0.01	3.82	**<0.001**
Sex	-0.28	0.14	-2.09	**0.04**
(Constant)	2.62	0.23	11.55	<0.001

B.2.4 GNB3 *C825T*

The model explained 2.4% of the variance of GDS-15 scores ($R^2 = 0.024$, $F(3,783) = 6.34$, $p < 0.001$). Age and sex significantly predicted GDS-15 scores ($B = 0.06$, $p < 0.001$ and $B = -0.28$, $p = 0.04$ respectively).

Table B.10. Multiple regression of *GNB3* C825T genotype in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
GNB3 C825T	-0.07	0.11	-0.64	0.53
Age (mean-centred)	0.06	0.01	3.85	**<0.001**
Sex	-0.28	0.14	-2.07	**0.04**
(Constant)	2.66	0.23	11.60	<0.001

B.2.5 MTHFR *C677T*

The model explained 2.4% of the variance of GDS-15 scores ($R^2 = 0.024$, $F(3,783) = 6.56$, $p < 0.001$). Age significantly predicted GDS-15 scores ($B = 0.05$, $p < 0.001$).

Table B.11. Multiple regression of *MTHFR* C677T genotype in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
MTHFR C677T	0.10	0.10	1.02	0.31
Age (mean-centred)	0.05	0.01	3.83	**<0.001**
Sex	-0.27	0.14	-1.95	0.05
(Constant)	2.52	0.25	10.31	<0.001

B.2.6 rs40465

The model explained 2.3% of the variance of GDS-15 scores ($R^2 = 0.023$, $F(3,783) = 6.20$, $p < 0.001$). Age and sex significantly predicted GDS-15 scores ($B = 0.05$, $p < 0.001$ and $B = -0.28$, $p = 0.04$ respectively).

Table B.12. Multiple regression of rs40465 genotype in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
rs40465	0.01	0.10	0.13	0.90
Age (mean-centred)	0.05	0.01	3.83	**<0.001**
Sex	-0.28	0.14	-2.09	**0.04**
(Constant)	2.62	0.23	11.26	<0.001

B.3 Secondary analyses for effects of early-life trauma on LLD using continuous GDS-15 score as the dependent variable

The main effects of childhood maltreatment and Holocaust trauma on GDS-15 scores (dependent variable) were investigated in two separate multiple regression models, with age and sex also included as predictors.

B.3.1 Childhood maltreatment

The model explained 12.3% of the variance of GDS-15 scores ($R^2 = 0.123$, $F(7,450) = 8.99$, $p < 0.001$). Age significantly predicted GDS-15 scores ($B = 0.15$, $p < 0.001$). Out of the five types of childhood maltreatment assessed in the CTQ-SF, both emotional abuse and sexual abuse significantly predicted GDS-15 scores ($B = 1.52$, $p < 0.001$ and $B = 1.08$, $p = 0.03$ respectively).

Table B.13. Multiple regression of childhood maltreatment in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
Emotional abuse	1.52	0.41	3.72	**<0.001**
Physical abuse	-0.51	0.42	-1.22	0.22
Sexual abuse	1.08	0.50	2.15	**0.03**
Emotional neglect	-0.32	0.41	-0.78	0.44
Physical neglect	0.27	0.43	0.62	0.54
Age (mean-centred)	0.15	0.02	6.37	**<0.001**
Sex	-0.04	0.21	-0.21	0.84
(Constant)	2.70	0.34	7.83	<0.001

B.3.2 Holocaust trauma

The model explained 9.5% of the variance of GDS-15 scores ($R^2 = 0.095$, $F(3,454) = 15.87$, $p < 0.001$). Age and exposure to Holocaust trauma significantly predicted GDS-15 scores ($B = 0.14$, $p < 0.001$ and $B = 1.04$, $p = 0.004$ respectively).

Table B.14. Multiple regression of Holocaust trauma in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
Holocaust trauma	1.04	0.36	2.91	**0.004**
Age (mean-centred)	0.14	0.02	6.03	**<0.001**
Sex	0.03	0.21	0.17	0.87
(Constant)	2.60	0.34	7.61	<0.001

B.4 Secondary analyses for gene-environment interactions on LLD using continuous GDS-15 score as the dependent variable

Potential gene-environment interactions between the candidate polymorphism of interest and childhood maltreatment or Holocaust trauma were tested in separate multiple regression models. Each model included the candidate polymorphism main effect, childhood maltreatment or Holocaust trauma main effect, the gene-environment interaction, as well as age and sex as predictors.

B.4.1 APOE *ε2 x childhood maltreatment*

The model explained 9.3% of the variance of GDS-15 scores ($R^2 = 0.093$, $F(5,452) = 9.28$, $p < 0.001$). Age and childhood maltreatment significantly predicted GDS-15 scores ($B = 0.15$, $p < 0.001$ and $B = 0.55$, $p = 0.04$ respectively).

Table B.15. Multiple regression of *APOE* ε2, childhood maltreatment and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
APOE ε2	-0.29	0.32	-0.89	0.37
Childhood maltreatment	0.55	0.26	2.11	**0.04**
APOE ε2 x childhood maltreatment	0.57	0.77	0.74	0.46
Age (mean-centred)	0.15	0.02	6.31	**<0.001**
Sex	0.01	0.21	0.05	0.96
(Constant)	2.64	0.35	7.60	<0.001

B.4.2 APOE *ε2 x Holocaust trauma*

The model explained 9.7% of the variance of GDS-15 scores ($R^2 = 0.097$, $F(5,452) = 9.71$, $p < 0.001$). Age and Holocaust trauma significantly predicted GDS-15 scores ($B = 0.14$, $p < 0.001$ and $B = 1.10$, $p = 0.01$ respectively).

Table B.16. Multiple regression of *APOE* ε2, Holocaust trauma and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
APOE ε2	-0.27	0.31	-0.87	0.39
Holocaust trauma	1.10	0.40	2.72	**0.01**
APOE ε2 x Holocaust trauma	-0.16	0.88	-0.18	0.86
Age (mean-centred)	0.14	0.02	5.97	**<0.001**
Sex	0.05	0.21	0.22	0.82
(Constant)	2.62	0.34	7.63	<0.001

B.4.3 APOE *ε4 x childhood maltreatment*

The model explained 9.5% of the variance of GDS-15 scores ($R^2 = 0.095$, $F(5,452) = 9.54$, $p < 0.001$). Age and childhood maltreatment significantly predicted GDS-15 scores ($B = 0.15$, $p < 0.001$ and $B = 0.55$, $p = 0.04$ respectively).

Table B.17. Multiple regression of *APOE* ε4, childhood maltreatment and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
APOE ε4	0.25	0.29	0.87	0.39
Childhood maltreatment	0.55	0.27	2.02	**0.04**
APOE ε4 x childhood maltreatment	0.39	0.62	0.63	0.53
Age (mean-centred)	0.15	0.02	6.39	**<0.001**
Sex	0.01	0.21	0.05	0.96
(Constant)	2.55	0.35	7.34	<0.001

B.4.4 APOE *ε4 x Holocaust trauma*

The model explained 10.2% of the variance of GDS-15 scores ($R^2 = 0.102$, $F(5,452) = 10.31$, $p < 0.001$). Age and Holocaust trauma significantly predicted GDS-15 scores ($B = 0.14$, $p < 0.001$ and $B = 0.81$, $p = 0.04$ respectively).

Table B.18. Multiple regression of *APOE* ε4, Holocaust trauma and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
APOE ε4	0.24	0.26	0.91	0.36
Holocaust trauma	0.81	0.39	2.07	**0.04**
APOE ε4 x Holocaust trauma	1.31	0.94	1.40	0.16
Age (mean-centred)	0.14	0.02	6.23	**<0.001**
Sex	0.01	0.21	0.05	0.96
(Constant)	2.60	0.35	7.51	<0.001

B.4.5 BDNF *Val66Met x childhood maltreatment*

The model explained 10.7% of the variance of GDS-15 scores ($R^2 = 0.107$, $F(5,452) = 10.79$, $p < 0.001$). Age and the *BDNF* Val66Met x childhood maltreatment interaction significantly predicted GDS-15 scores ($B = 0.14$, $p < 0.001$ and $B = 1.19$, $p = 0.01$ respectively).

Table B.19. Multiple regression of *BDNF* Val66Met, childhood maltreatment and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
BDNF Val66Met	-0.33	0.21	-1.53	0.13
Childhood maltreatment	0.17	0.29	0.58	0.56
BDNF Val66Met x childhood maltreatment	1.19	0.43	2.79	**0.01**
Age (mean-centred)	0.14	0.02	6.30	**<0.001**
Sex	-0.01	0.20	-0.04	0.97
(Constant)	2.75	0.35	7.81	<0.001

B.4.6 BDNF *Val66Met x Holocaust trauma*

The model explained 9.6% of the variance of GDS-15 scores ($R^2 = 0.096$, $F(5,452) = 9.63$, $p < 0.001$). Age and Holocaust trauma significantly predicted GDS-15 scores ($B = 0.14$, $p < 0.001$ and $B = 0.89$, $p = 0.03$ respectively).

Table B.20. Multiple regression of *BDNF* Val66Met, Holocaust trauma and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
BDNF Val66Met	-0.04	0.20	-0.20	0.84
Holocaust trauma	0.89	0.40	2.21	**0.03**
BDNF Val66Met x Holocaust trauma	0.54	0.66	0.83	0.41
Age (mean-centred)	0.14	0.02	5.98	**<0.001**
Sex	0.03	0.21	0.15	0.88
(Constant)	2.62	0.35	7.48	<0.001

B.4.7 GNB3 *C825T x childhood maltreatment*

The model explained 9.2% of the variance of GDS-15 scores ($R^2 = 0.092$, $F(5,452) = 9.13$, $p < 0.001$). Age significantly predicted GDS-15 scores ($B = 0.15$, $p < 0.001$).

Table B.21. Multiple regression of *GNB3* C825T, childhood maltreatment and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
GNB3 C825T	-0.08	0.19	-0.41	0.68
Childhood maltreatment	0.64	0.34	1.87	0.06
GNB3 C825T x childhood maltreatment	-0.03	0.39	-0.08	0.93
Age (mean-centred)	0.15	0.02	6.34	**<0.001**
Sex	0.01	0.21	0.03	0.98
(Constant)	2.65	0.37	7.18	<0.001

B.4.8 GNB3 *C825T x Holocaust trauma*

The model explained 9.9% of the variance of GDS-15 scores ($R^2 = 0.099$, $F(5,452) = 9.93$, $p < 0.001$). Age and Holocaust trauma significantly predicted GDS-15 scores ($B = 0.14$, $p < 0.001$ and $B = 1.63$, $p = 0.01$ respectively).

Table B.22. Multiple regression of *GNB3* C825T, Holocaust trauma and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
GNB3 C825T	-0.07	0.17	-0.41	0.68
Holocaust trauma	1.63	0.60	2.71	**0.01**
GNB3 C825T x Holocaust trauma	-0.75	0.63	-1.20	0.23
Age (mean-centred)	0.14	0.02	5.92	**<0.001**
Sex	0.03	0.21	0.17	0.87
(Constant)	2.65	0.36	7.30	<0.001

B.4.9 MTHFR *C677T x childhood maltreatment*

The model explained 11.0% of the variance of GDS-15 scores ($R^2 = 0.11$, $F(5,452) = 11.15$, $p < 0.001$). Age significantly predicted GDS-15 scores ($B = 0.14$, $p < 0.001$).

Table B.23. Multiple regression of *MTHFR* C677T, childhood maltreatment and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
MTHFR C677T	0.26	0.16	1.67	0.10
Childhood maltreatment	0.29	0.35	0.82	0.41
MTHFR C677T x childhood maltreatment	0.53	0.35	1.55	0.12
Age (mean-centred)	0.14	0.02	6.33	**<0.001**
Sex	0.06	0.21	0.29	0.77
(Constant)	2.29	0.38	6.02	<0.001

B.4.10 MTHFR *C677T x Holocaust trauma*

The model explained 10.3% of the variance of GDS-15 scores ($R^2 = 0.103$, $F(5,452) = 10.34$, $p < 0.001$). Age significantly predicted GDS-15 scores ($B = 0.14$, $p < 0.001$).

Table B.24. Multiple regression of *MTHFR* C677T, Holocaust trauma and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
MTHFR C677T	0.27	0.15	1.79	0.08
Holocaust trauma	0.76	0.69	1.10	0.27
MTHFR C677T x Holocaust trauma	0.14	0.50	0.28	0.78
Age (mean-centred)	0.14	0.02	5.96	**<0.001**
Sex	0.07	0.21	0.35	0.73
(Constant)	2.33	0.37	6.29	<0.001

B.4.11 rs40465 x childhood maltreatment

The model explained 9.3% of the variance of GDS-15 scores ($R^2 = 0.093$, $F(5,452) = 9.24$, $p < 0.001$). Age significantly predicted GDS-15 scores ($B = 0.15$, $p < 0.001$).

Table B.25. Multiple regression of rs40465, childhood maltreatment and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
rs40465	0.10	0.18	0.58	0.56
Childhood maltreatment	0.55	0.34	1.63	0.11
rs40465 x childhood maltreatment	0.11	0.36	0.31	0.75
Age (mean-centred)	0.15	0.02	6.36	**<0.001**
Sex	0.01	0.21	0.06	0.96
(Constant)	2.53	0.36	7.02	<0.001

B.4.12 rs40465 x Holocaust trauma

The model explained 10.2% of the variance of GDS-15 scores ($R^2 = 0.102$, $F(5,452) = 10.22$, $p < 0.001$). Age and Holocaust trauma significantly predicted GDS-15 scores ($B = 0.14$, $p < 0.001$ and $B = 1.83$, $p = 0.002$ respectively).

Table B.26. Multiple regression of rs40465, Holocaust trauma and their interaction in predicting GDS-15 scores.

Predictor	*B*	*SE*	*t*	*p*
rs40465	0.17	0.16	1.05	0.29
Holocaust trauma	1.83	0.59	3.10	**0.002**
rs40465 x Holocaust trauma	-1.02	0.59	-1.73	0.09
Age (mean-centred)	0.14	0.02	6.04	**<0.001**
Sex	0.04	0.21	0.19	0.85
(Constant)	2.49	0.36	7.00	<0.001

APPENDIX C. PUBLISHED MANUSCRIPT

Tsang, R. S. M., Mather, K. A., Sachdev, P. S., & Reppermund, S. (2017). Systematic review and meta-analysis of genetic studies of late-life depression. *Neuroscience & Biobehavioral Reviews*, 75, 129-139.