Genetic Epidemiology of Psychotic Symptoms and their Treatment in Dementia

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Genetic Epidemiology of Psychotic Symptoms and their Treatment in Dementia

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Abstract

There is an urgent need to develop safe and effective treatments for psychotic symptoms in dementia and make better use of current treatments. This will be aided by a better understanding of the mechanisms underlying both the symptoms and treatments. Thus, two lines of research were followed: 1) analysis of the prevalence and course of psychotic symptoms in Alzheimer's disease (AD) and dementia with Lewy bodies (DLB)/Parkinson's disease dementia (PDD), and, 2) analysis of the response to treatments in AD. To address the former, the *COMT* val158met and 5HTTLPR polymorphisms were assessed with respect to the presence of persistent symptoms and the *MAPT* haplotype with respect to the course of symptoms. The 5HTTLPR LL genotype was associated with a significantly increased risk of persistent delusions in DLB/PDD and the *MAPT* haplotype with a significantly increased risk of worsening delusions in AD. Finally, the *COMT* val158met polymorphism was found to predict more rapid cognitive decline in mild AD patients.

The examination of point 2) above chiefly concerned histamine H1 receptor antagonism and antipsychotic mortality. Patients taking high affinity H1 antipsychotics had a significantly greater mortality risk compared with those taking no antipsychotics with evidence also suggesting effect modification by the *HNMT* gene polymorphism.

Collectively, in what are among the best characterised cohorts to date, these findings bring greater clarity to the current understanding of the role of 5HTTLPR and *COMT* val158met in contributing to psychotic symptoms in dementia, supporting the prioritisation of serotonin-acting treatments in DLB/PDD and suggesting that dopamine levels may indirectly influence the presentation of psychosis via more rapid cognitive decline in AD. New evidence was also found to support the hypotheses that tau pathology is associated with psychosis and histamine antagonism is a key harmful property of antipsychotics, with important implications for treatment strategies.

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List of Abbreviations

5-HT	5-hydroxytryptamine, serotonin
5HTIAA	5-Hydroxyindoleacetic acid
5HTT	Serotonin transporter
ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ADL	Activities of Daily Living
ANOVA	Analysis of variance
APOE	Apolipoprotein E
APP	Amyloid precursor protein
Aβ	Beta-amyloid peptide
bp	Base pair
CAMDEX	Cambridge Mental Disorders of the Elderly Examination
CDR	Clinical Dementia Rating Scale
CERAD	Consortium to Establish a Registry for Alzheimer’s Disease
ChAT	Choline acetyltransferase
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CSF	Cerebrospinal fluid
CUSPAD	Columbia University Scale to Assess Psychopathology in Alzheimer's Disease
DA	Dopamine
DAT	Dopamine transporter
DeNDRoN	Dementia and Neurodegenerative Diseases Research Network
DLB	Dementia with Lewy bodies
DLPFC	Dorsolateral prefrontal cortex
dNTP	Deoxyribonucleotide triphosphate
DRN	Dorsal raphe nucleus
DSM	Diagnostic and Statistical Manual of Mental Disorders
dUTP	Diphosphatase
EDTA	Ethylenediaminetetraacetic acid
FAST	Functional Assessment Staging Tool
FTD	Frontotemporal dementia
GWAS	Genome wide association study
H1AP-	Low/no affinity H1 antipsychotic
H1AP+	High affinity H1 antipsychotic
HNMT	Histamine-N-methyltransferase
HR	Hazard ratio
Ile	Isoleucine
LB	Lewy body
MAF	Minor allele frequency
MAPT	Microtubule associated protein tau
Met	Methionine
MHRN	Mental Health Research Network
MMSE	Mini mental state examination
MRN	Medial raphe nucleus
mRNA	messenger RNA

NFT	Neurofibrillary tangles
NIA-REAGAN	National Institute on Aging and the Ronald and Nancy Reagan Institute of the Alzheimer's Association
NINCDS-ADRDA	Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NMDAR	N-metyl-D-aspartate receptor
NPI	Neuropsychiatric Inventory
NTC	Non template control
OR	Odds ratio
PBE	Present Behavioural Examination
PCR	Polymerase chain reaction
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PET	Positron emission tomography
PFC	Prefrontal cortex
PRN	Pro ne rata, as required
PSP	Progressive supranuclear palsy
RCT	Randomised controlled trial
REC	Research Ethics Committee
SD	Standard deviation
SLC6A4	Solute carrier 6 member 4
SN	Substania nigra
SNP	Single nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitor
Thr	Threonine
VaD	Vascular dementia
Val	Valine
VNTR	Variable number tandem repeat

Acknowledgments

Firstly, I would like to thank both of my supervisors, Professor Clive Ballard and Dr Emma Jones for giving the opportunity to undertake this project. It was a pleasure to come back to science after nearly three years in the city and I cannot think of a subject and project I'd rather have worked on. Thank you for your guidance and advice throughout and in particular the opportunity to be involved with such a diverse range of research activity. It has become plainly obvious to me that these sorts of studies are not possible without large cross centre collaborations so I would like to thank all of the contributors of clinical data and samples. Particular thanks to Dr John Powell for the lab space and Megan Pritchard for the desk space at the IOP. And when speaking of contributors, it is only right to reserve the biggest thanks to the participants and patients, and their families, for supporting our work.

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Declaration

Chapter 1 General Introduction

Related papers:

- Creese, B., Ballard, C., Jones, E. (2013) Cognitive Impairment in Studies of 5HTTLPR and Psychosis in Alzheimer's Disease: A Systematic Review. *Dement Geriatr Cogn Disord*. 35(3-4), 155-164.
- Corbett, A., Smith, J., Creese, B., Ballard, C. (2012) *Treatment of Behavioural and Psychological Symptoms in Alzheimer's Disease*. *Curr Treat Options Neurol.* 14(2), 113-125.
- Ballard, C., Creese, B., Corbett, A., Aarsland, D. (2011) Atypical Antipsychotics for the Treatment of Behavioural and Psychological Symptoms in Dementia, with a Particular Focus on Longer Term Outcomes and Mortality. *Expert Opin Drug Saf.* 10(1) , 35-43

1.1 Clinical features, diagnoses and pathology of Alzheimer's disease and dementia with Lewy bodies/Parkinson's disease dementia

1.1.1 Overview and clinical diagnoses of dementia

It is estimated that 34 million people have dementia worldwide. Alzheimer's disease (AD) is the most common form of dementia, accounting for around 62% of cases while Lewy body dementias (dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD)) account for around 15% of patients. Broadly, AD, DLB and PDD are insidious in their onset and characterised by the central clinical feature of progressive cognitive impairment. PD on the other hand is characterised clinically by movement disorders such as rigidity and tremor and although it is not characterised by dementia per se, the majority of people with PD will go on to develop PDD (Aarsland, Andersen et al. 2003). The vast majority of patients with AD, DLB and PDD have the sporadic form of the disease, with causes thought to arise from a variety of genetic and environmental sources (Lesage, Brice 2009, Chartier-Harlin, Crawford et al. 1991, Zarranz, Alegre et al. 2004, Zarranz, Alegre et al. 2004).

The key differences between AD and DLB that confer the greatest reliability in making a differential diagnosis have been incorporated in operationalised diagnostic criteria (a diagnosis of PDD is made when dementia develops in the context of established PD, this will be returned to later in this section). These criteria are the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for AD and the consensus criteria set out by McKeith et al. (2005) for DLB first established in 1984 and 1996 respectively (McKeith, Dickson et al. 2005, Dubois, Feldman et al. 2007, McKhann, Drachman et al. 1984, McKeith, Galasko et al. 1996). Both of these have undergone subsequent revisions (McKhann, Knopman et al. 2011, McKeith, Dickson et al. 2005) to incorporate advances in biomarker identification and to improve early differential diagnosis (Ballard, Halliday 2010). In particular the revision to the NINCDS-ADRDA criteria places a good deal of emphasis on the pathophysiological and clinically observable characteristics of AD, focusing on defining AD in the pre-clinical, pre-dementia and full-blown disease stages (Jack, Albert et al. 2011, Sperling, Aisen et al. 2011, Albert, DeKosky et al. 2011). Although any patients

with a diagnosis of probable AD from the 1984 criteria would fall under probable AD in the 2011 criteria, possible AD patients under the 1984 criteria would not necessarily fall under the possible AD diagnosis of the 2011 criteria. This is in part due to a greater recognition of DLB in the years following the 1984 criteria and the recognition that negative results of certain biomarker tests (positron emission tomography (PET) imaging of amyloid-β, cerebrospinal fluid (CSF) tau, structural magnetic resonance imaging) are not compatible with a diagnosis of AD regardless of clinical criteria being met. The latest NINCDS-ADRDA criteria were introduced too late for use in the cohorts used with the present project therefore the 1984 criteria will be discussed henceforth, unless otherwise specified.

In 2007 Dubois et al. developed research criteria for diagnosing AD, based on the NINCDS-ADRDA criteria, which was followed by the updated AD lexicon in 2010. (Dubois, Feldman et al. 2007, Dubois, Feldman et al. 2010). Both of these place greater emphasis on the pre-dementia stages of early episodic memory impairment supported by biomarker confirmation, incorporating them into the diagnostic label of 'AD'. The term 'AD dementia' is proposed and refers to stages of AD where cognitive symptoms are severe enough to interfere with daily functioning.

Another widely used tool for the clinical diagnosis of dementia is the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria published by the American Psychiatric Association. The most recent edition (DSM-5) was published in May 2013 and contains revisions of terminology such that the former diagnostic codes relating to dementia have been replaced by codes referring to 'major and mild neurocognitive disorders'. It is important to note that this latest edition now includes specific criteria for dementia with Lewy bodies ('Major or Mild Neurocognitive Disorder with Lewy Bodies').

The most recent DSM edition on which diagnoses were made in this project was the DSM-IV. Though there have been two more recent editions the criteria for dementia of any kind have not changed significantly (American Psychiatric Association. and American Psychiatric Association. Task Force on DSM-IV 1994). At the time samples were collected for this project there were no DSM criteria for DLB and the criteria for PDD was classified under 'dementia due to other medical conditions'. Indeed, because of this the McKeith criteria for DLB have long been the principal criteria for the diagnosis of DLB. The clinical diagnosis of PDD is a little less straightforward as

there has only been one set of criteria specifically proposed which have yet to be validated (Emre, Aarsland et al. 2007). The diagnosis of PDD will be returned to later in this section.

The DSM edition includes criteria for AD; those that form the DSM-IV (appendix A) are commonly used by clinicians to diagnose the disease. However, the NINCDS-ADRDA criteria which offer greater validity and reliability, are more widely used for research purposes and so will be the focus of the remaining discussion concerning the clinical diagnosis of AD.

The NINCDS-ADRDA and McKeith criteria themselves differ slightly in their categorisation but broadly speaking there are those items that are essential for a diagnosis of the dementia in question, those that are consistent or supportive with that diagnosis and those whose presence is not compatible with the diagnosis or make diagnosis less likely. The combination of the different levels of criteria then determines whether the diagnosis is probable or possible.

The NINCDS-ADRDA and McKeith criteria are listed in full in appendix B and appendix C respectively. In both cases progressive cognitive impairment must be present for a diagnosis to be made. However, the central cognitive criteria in both have been developed as a result of observations from research that deficits in specific cognitive domains, rather than global measures, offer the best differentiation between AD and DLB. Typically, patients with DLB present with greater deficits in visuoperceptual ability, attention and executive function than AD patients, who are generally more impaired on tasks associated with hippocampal function, such as verbal and episodic memory (Tiraboschi 2006, Noe, Marder et al. 2004, Aarsland 2003, Collerton, Burn et al. 2003, Noe, Marder et al. 2004). Longitudinal evaluation of symptoms is central to an accurate diagnosis in both dementias. “Progressive worsening” is a prerequisite for AD according to the NINCDS-ADRDA criteria, and as would be expected there is much more of a focus on executive and attentional deficits among the central features of DLB, while memory impairment “is usually evident with progression” but does not have to be persistent or prominent in the early stages, unlike in AD (McKeith, Dickson et al. 2005).

Neuropsychiatric symptoms, while common in the more moderate and advanced disease stages, do not form part of the diagnostic criteria for AD. In keeping with the consistent evidence that visual hallucinations are present in the majority of patients with DLB (Aarsland, Ballard et al.

2001) this symptom forms one of the core features of the McKeith criteria for DLB, the others being fluctuating cognition and early Parkinsonism. According to the McKeith criteria, a diagnosis of probable DLB can be made on the basis of the central cognitive features described in the above paragraph plus at least two of these core features. Alternatively, the central features and one core feature coupled with one or more of the suggestive features (REM sleep disorder, neuroleptic sensitivity and low dopamine transporter uptake) can be used to diagnose probable DLB. Central and suggestive features only (in the absence of any core features) can only lead to a diagnosis of possible DLB (see appendix C).

Under the NINCDS-ADRDA criteria, a diagnosis of probable AD can be made when the criteria under section 1 (appendix B) are met, while a diagnosis of possible AD is generally reserved for otherwise unspecified dementia syndromes. For research purposes this may be a "single gradually progressive severe cognitive deficit in the absence of other identifiable causes." A diagnosis of definite AD is only possible after neuropathological examination (see section 1.1.2 for neuropathology of AD).

Concurrent cerebrovascular disease (which may cause a sudden onset of dementia or be evident from hemiparesis, visual field loss or brain imaging) is an important exclusion criterion for both DLB and AD. Furthermore, as alluded to above, early Parkinsonism is not compatible with AD and in DLB the onset of Parkinsonism is important in the clinical differentiation between it and PDD.

Clinically and pathologically, there is little that separates DLB from PDD. A diagnosis of PDD is made when the symptoms of dementia occur in the context of established PD. For research purposes the threshold is set at an arbitrary 1 year. That is, if symptoms of dementia occur more than one year after the onset of Parkinsonism then a diagnosis of PDD is made (McKeith, Dickson et al. 2005). PD is diagnosed according to the UK Brain Bank criteria, consisting of three steps (full details are included in appendix D) (Hughes, Daniel et al. 1992). Step one involves diagnosing a Parkinsonian syndrome which must consist of bradykinesia as well as rigidity, resting tremor or postural instability not caused by dysfunction in other domains. Step two requires the meeting of a series of exclusion criteria, along with three or more of the supportive features of step three in order for a diagnosis of definite PD to be made. With regard to then

diagnosing dementia (i.e. PDD), a common procedure is to diagnose PD according to the Queen Square criteria. Then if dementia, according to the DSM-IV (appendix A) or other appropriate criteria, presents more than one year after the onset of PD a diagnosis of PDD is made. Diagnosing PDD by the Queen Square criteria and DSM-IV has been shown to have good positive predictive value in prospective validation studies. Although more detailed criteria for PDD have been proposed (Emre, Aarsland et al. 2007) they have yet to be validated.

The one year threshold is useful in the clinic but there is in fact little evidence that DLB and PDD are two separate entities. Indeed, most research is supportive of a spectrum view where both DLB and PDD (collectively Lewy body (LB) dementias) and PD fall on a continuum of Lewy body disease.

In keeping with the continuum view, there appears to be few differences in cognitive impairment in PDD and DLB. Individuals with mild DLB have been shown to exhibit greater impairments in some, but not all, measures of executive function than PDD (Aarsland 2003). However these differences do not appear to be present among patients with severe dementia while other studies have failed to find any difference at all between the two in a wide variety of cognitive domains (Noe, Marder et al. 2004). Furthermore, although visual hallucinations are more common in DLB than PDD, the content and severity are identical (Aarsland, Ballard et al. 2001, Mosimann, Rowan et al. 2006). There is also no way of distinguishing between the two diseases based on other neuropsychiatric symptoms.

1.1.2 Pathology and pathological diagnoses of dementia

DLB and PDD are also characterised by very similar underlying pathology. In both cases cortical Lewy body (LB) pathology is the hallmark feature, particularly in the frontal, cingulate and entorhinal cortices. Lewy bodies are abnormal protein aggregates with α-synuclein being the core component in both DLB/PDD and PD (Spillantini, Schmidt et al. 1997), but it is their presence in the cortex that is strongly associated with the development of dementia. Specifically, Hurtig et al. (2000) used a LB staging system in a variety of brain areas including the (amygdala, hippocampus, subiculum, entorhinal cortex, midfrontal gyrus, postcentral gyrus, Wernicke's area and cingulate gyrus) ranging from 0 to 3+, with 0 being no LBs and 3+ being >=5 per 100x field

(Hurtig, Trojanowski et al. 2000). They found good sensitivity and specificity, with 91% of patients diagnosed with PDD clinically being found to have a cortical LB score of 2 or more and 90% of patients diagnosed with PD only having a cortical LB score of less than 2. Consistent with this finding, cortical LB counts in PDD and also DLB brains were found to be significantly higher than in PD in a later study, whereas a greater proportion of brainstem LB pathology was associated with PD (Tsuboi, Dickson 2005). Of note, the authors did not report any significant difference between the number and distribution of LB pathology in DLB and PDD, a finding which has also been observed in imaging analysis of grey matter atrophy (Burton 2004).

The close clinical and pathological similarity between DLB and PDD has led most experts to support the LB disease continuum view, making the grouping together of these two syndromes acceptable for the study of underlying disease mechanisms (Aarsland, Ballard et al. 2004, McKeith, Dickson et al. 2005, McKeith 2007). Besides Lewy body pathology, the brains of DLB/PDD patients frequently show the two hallmark pathological features of AD pathology: amyloid plaques and neurofibrillary tangles (NFT). Tsuboi and Dickson (2005) examined the presence of AD pathology in the cortex and hippocampus of DLB patients and found NFTs to be completely absent in the former region while both amyloid plaques and NFTs were present in the latter (Tsuboi, Dickson 2005)(McKeith, Dickson et al. 2005)(McKeith, Dickson et al. 2005)(McKeith, Dickson et al. 2005)(McKeith, Dickson et al. 2005)(McKeith, Dickson et al. 2005)(McKeith, Dickson et al. 2005)(McKeith, Dickson et al. 2005)(McKeith, Dickson et al. 2005)(McKeith, Dickson et al. 2005). Similarly, in a later study significantly more amyloid plaques were found in the cortex and hippocampus, and significantly more NFTs found in the hippocampus, in DLB and PDD compared to PD, while the levels in DLB and PDD were not different (Tsuboi, Uchikado et al. 2007). The McKeith diagnostic criteria take account of this and post-mortem cases are assigned a likelihood that the dementia of the patient was due to AD pathology.

As stated above, the two hallmark pathological lesions associated with AD are amyloid plaques and NFTs. NFTs are chiefly composed of abnormally phosphorylated and hyperphosphorylated tau protein while amyloid plaques are defined by the presence of amyloid β-peptide (Aβ) (Masters, Simms et al. 1985).

In addition to being the major component of NFTs in AD, there are pathological lesions characterised by abnormally phosphorylated and hyperphosphorylated tau protein present in other diseases, collectively known as tauopathies. Alongside AD there are at least 25 such diseases, including Pick's disease and other forms of frontotemporal dementia (FTD), progressive supranuclear palsy (PSP) and corticobasal degeneration (Spillantini, Goedert 2013). The tau protein is encoded by a single gene composed of 16 exons, microtubule-associated protein tau (*MAPT*), on chromosome 17. There are six isoforms in the adult human brain, generated by alternative splicing of exons 2, 3 and 10. As shown in Figure 1-1, exons 2 and 3, just exon 2 or neither can be included (2N, 1N or 0N respectively). Additionally, in each case a 31 amino acid repeat (exon 10) can either be omitted or included, generating three 3-repeat (3R) isoforms and three 4-repeat (4R) isoforms.

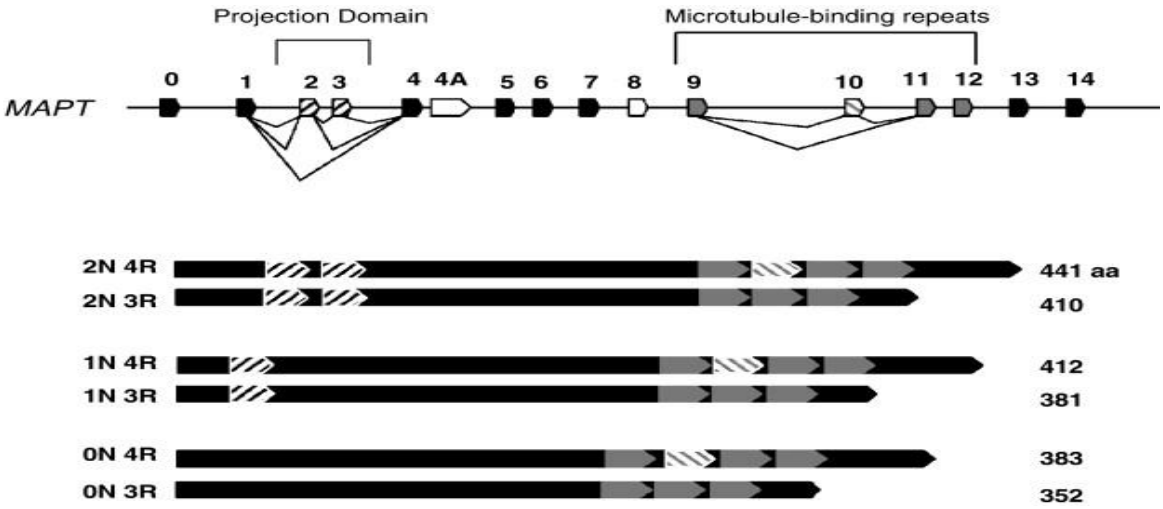

Figure 1-1 *MAPT* isoforms

In the MAPT gene exons 2, 3 and 10 are alternatively spliced to generate the six isoforms present in the human brain. Figure from (Caffrey, Wade-Martins 2007)

There are approximately equal amounts of 3R and 4R tau in the healthy adult human cortex but aggregation of either of the isoforms into filamentous inclusions is associated with various tauopathies. For example, NFTs in AD contain all six isoforms, although some research suggests there is more 3R than 4R (Spillantini, Goedert 2013, Liu, Le et al. 2001) while tau filaments in PSP and corticobasal degeneration both contain only the 4R isoform and in Pick's disease they are composed only of the 3R isoform (Spillantini, Goedert 2013).

Aβ is produced by cleavage of the amyloid precursor protein (*APP* gene on chromosome 21) and exists in varying lengths usually ranging from 38 to 43 amino acids (Ritchie, McLean et al. 2010). $A\beta_{42}$ is the major component of amyloid plaques in AD (Iwatsubo, Odaka et al. 1994).

The amyloid cascade hypothesis has provided the prevailing model of neurodegeneration in AD (Hardy, Allsop 1991, Hardy, Higgins 1992). This hypothesis originally put amyloid deposition as the first pathological feature of AD, caused by increases in total Aβ, increases in the ratio of $A\beta_{42}$:$A\beta_{4o}$ and decreased Aβ degradation and clearance. The formation of plaques is followed by NFTs (although the mechanism linking the two is not clear), thus according to this hypothesis the cell death that follows in turn is either a direct result of amyloid deposition, caused indirectly via NFTs, or both. Central to the formation and support of this hypothesis is genetic evidence that mutations in the *APP* gene cause early onset familial AD and are associated with increases in either total Aβ or the $A\beta_{42}$:$A\beta_{4o}$ ratio. Moreover, the *APP* gene is located on chromosome 21 and in people with Down syndrome it is therefore triplicated. Both amyloid plaques and NFTs are present in the majority of people with Down syndrome by age 50. They form in a pattern typically observed in the wider AD population (Mann, Esiri 1989), with NFTs developing later and being better correlated with the clinically observed severity of dementia (Margallo-Lana, Moore et al. 2007), which is also true of AD patients without Down syndrome (Thind, Sabbagh 2007). Following on from the original hypothesis and from evidence indicating that amyloid plaques are not correlated with dementia severity, research focused on smaller soluble Aβ dimers, trimers and oligomers. Specifically, a range of neurotoxic events may be associated with these smaller units of Aβ in people with AD, resulting from both their intra- and extracellular accumulation. Intracellular neurotoxic events may arise from the reuptake of Aβ at several different receptors, potentially interfering with the proteasome. Moreover, extracellular neurotoxicity has been proposed to arise from Aβ binding at several receptors, including NMDA and frizzled receptors which may lead to cell death via mitochondrial dysfunction and wnt signalling respectively. The latter providing one explanation for how Aβ may cause NFT formation (reviewed in (Kayed, Lasagna-Reeves 2013).

It is important to highlight that although AD is characterised by both NFTs and amyloid plaques, they do not encompass the full extent of the neurological pathology associated with the disease. Mitochondrial dysfunction, oxidative stress and neuroinflammation are also implicated in the

pathogenesis of AD (Serrano-Pozo, Frosch et al. 2011). Although the amyloid cascade hypothesis would put amyloid deposition as the starting point for all of these, it should also be noted that a mitochondrial cascade hypothesis has also been proposed, perhaps reflecting an increased recognition of other pathological processes (Swerdlow, Khan 2004).

The progression and distribution of NFTs and amyloid plaques is predictable and for this reason various staging methods based on the severity of these lesions have been developed. For research purposes they are used as an indication of disease severity rather than as a full characterisation of the pathology present in the brain.

The NFT staging proposed by Braak and Braak (1991) is determined by the pattern and severity of burden and comprises of six stages. NFTs are mainly localised to the transentorhinal region in stages I-II, becoming more severe and involving the entorhinal cortex and limbic regions in the middle stages, III-IV. In the last stages (V-VI) NFT burden is still most severe in the aforementioned areas but overall there is significant involvement of most of the neocortex (Braak, Braak 1991). Amyloid deposition was also staged by Braak and Braak in the same paper, although the staging does not refer specifically to amyloid plaques as it includes diffuse amyloid deposition as well. First affected are basal areas of the frontal, temporal and occipital cortices. Amyloid deposition then spreads further to cover the rest of the neocortex (with the exception of the motor and sensory cortices) with the aforementioned basal areas still being the more severely affected. There is also mild deposition in the hippocampus. Finally, almost all areas of the neocortex become seriously affected and while there is a relative sparing of the hippocampus as a whole, there are areas of tightly packed amyloid deposits in CA1 and the fascia dentata. These three stages correspond to stages A, B and C defined by the authors (Braak, Braak 1991).

While the Braak and Braak staging focuses on the progression of NFT pathology the CERAD (Consortium to Establish a Registry for Alzheimer's Disease) neuropathological criteria are based on the severity (semi-quantitatively graded as none, sparse, moderate or frequent) of amyloid plaque pathology from three areas of the neocortex: the middle frontal gyrus, inferior parietal lobule and the superior and middle temporal gyri (Mirra, Heyman et al. 1991). The patient's age is then used alongside the plaque grade, giving an age-related plaque score of 0 (no histologic evidence of AD), A (uncertain evidence of AD), B (suggestive evidence of AD) or C (histologic

evidence indicates AD). This score is then integrated with the patient's clinical history, forming the final diagnostic criteria for AD: normal (i.e. no evidence of dementia), possible, probable or definite. These categories are based on the pathological criteria (the clinical history of dementia is not operationalised) and are not to be confused with the NINCDS-ADRDA criteria of the same names.

The National Institute on Aging and the Ronald and Nancy Reagan Institute of the Alzheimer's Association (NIA-REAGAN) staging considers all pathology in the brain to be abnormal, regardless of the clinical presentation of the patient during life. Based on the observed pathology the likelihood that AD accounts for the dementia is rated as high (amyloid plaques and NFTs in the neocortex), intermediate (moderate amyloid plaques and NFTs in limbic regions) or low (amyloid plaques and NFTs in a limited distribution and/or severity). These three stages correspond to Braak Stage V-IV/CERAD frequent score, Braak Stage III-IV/CERAD moderate and Braak Stage I-II/CERAD infrequent respectively.

Currently the only treatments available for dementia target symptoms, rather than underlying pathology, and although the amyloid cascade hypothesis has allowed the identification of amyloid deposition as a potential treatment target for AD, clinical trials in this area have yet to be successful (Lobello, Ryan et al. 2012). Effectively managing dementia and providing care for those with the disease is complicated by neuropsychiatric symptoms, particularly psychotic symptoms, which are common in all forms of dementia.

1.2 Psychotic symptoms in dementia

Besides visual hallucinations which characterise DLB/PDD clinically, a wide range of neuropsychiatric symptoms are present across all dementias, including AD. These include hallucinations, delusions, delusional misidentification, agitation/aggression, depression and sleep disturbances. Psychotic symptoms in all dementias are debilitating, persistent and are associated with a negative impact on disease course and often influence the decision to move patients into residential or nursing care (Lopez 2013, Stern, Albert et al. 1994).

Understanding psychosis in dementia is of central importance principally because there are currently no effective treatments (see section 1.2.3) but also because of its relationship with disease course which along with the nosology of the symptoms will be reviewed below.

Broadly, psychosis refers to two particular symptoms: delusions and hallucinations. Statistical data reduction techniques in a range of dementia disorders reliably identify a psychotic syndrome. In all instances this comprises delusions and hallucinations but along with these the psychosis syndrome is sometimes also composed of other symptoms such as anxiety and irritability (Proitsi, Hamilton et al. 2011, Levy, Cummings et al. 1996, Aalten, de Vugt et al. 2003, Aarsland, Larsen et al. 1999, Lange, Hopp et al. 2004, Lange, Hopp et al. 2004). Only delusions and hallucinations are considered here as these are the principal symptoms of psychosis, thus henceforth the terms psychosis or psychotic symptoms will refer to only these two symptoms.

In contrast to schizophrenia, hallucinations in dementia are mostly visual. Auditory hallucinations have been estimated to occur in around 10% of people with AD, and around 20-40% of people with DLB and PDD (Ropacki, Jeste 2005, Noe, Marder et al. 2004, Aarsland, Ballard et al. 2001). Auditory hallucinations most frequently occur with visual hallucinations, there are not many estimates of their prevalence in isolation but two studies in PD put that figure at 0 and 2% respectively (Jeste, Finkel 2000, Ropacki, Jeste 2005, Inzelberg, Kipervasser et al. 1998, Fenelon, Mahieux et al. 2000, Noe, Marder et al. 2004). It is not apparent from most of the genetic studies of psychosis in dementia what proportion of participants have auditory hallucinations, probably because most rating scales give a total hallucination score which will comprise all types.

Again in contrast to schizophrenia, delusions of grandeur or other complex delusions are rare in all types of dementia. The most common forms of delusion are paranoid types and misidentifications which are most frequently simple non-systematised beliefs concerning theft, harm and spousal infidelity (Jeste, Finkel 2000, Aarsland, Ballard et al. 2001). There is also no distinction between these types of delusion in most genetic studies, for the same reasons as given above, therefore henceforth the terms delusions and hallucinations will refer to all types of each unless otherwise specified.

Long-term follow up studies put the frequency of psychotic symptoms in AD at 30-50%, with delusions being more common than hallucinations (Craig, Mirakhur et al. 2005, Levy, Cummings et al. 1996, Aalten, de Vugt et al. 2005). In contrast, hallucinations are more common than delusions in DLB/PDD. In DLB delusions and hallucinations are reliably found to be present in a majority of people, around 60% and 70% respectively, while the frequencies are less in PDD at around 30% and 50% respectively (Aarsland, Bronnick et al. 2007, Ballard, Holmes et al. 1999, Aarsland, Ballard et al. 2001).

It is important to note that point prevalence estimates tend to underestimate the true frequency of psychotic symptoms. In the Aalten et al. (2005) study of 199 mostly AD patients (two and 19 had DLB or mixed dementia respectively), point prevalence was 31% for delusions and 11% for hallucinations. However, the two year cumulative prevalence was nearly double for hallucinations (20%) and rose considerably to 48% for delusions. Similarly the 30-50% figure above from the Craig et al. (2005) study is a long-term retrospective estimate and considerably higher than the point prevalence estimate of 35% for delusions and 20% for hallucinations. Similar patterns are observed in DLB; Ballard et al. (1999) report frequencies of delusions and hallucinations at first presentation to be 60% and 65% respectively, with those numbers rising to 70% and 73% for prevalence over the whole disease course.

The fact that symptom estimates for delusions and hallucinations tend to increase with prospective evaluation suggests there is a reasonably large number of people who develop the symptom during follow up, that is to say psychotic symptoms have a high incidence. Consistent with this, the one year incidence in AD reported by Levy et al. (1996) was 25% for psychosis. Aalten et al. (2005) calculated the 18 month incidence for delusions and hallucinations to be 34% and 20% respectively. Once again, similar figures are reported in DLB: 30% for both delusions and hallucinations (Ballard, O'Brien et al. 2001).

Psychotic symptoms are also persistent. Estimates of persistence vary somewhat due to different lengths of follow up but most findings suggest that once a psychotic symptom emerges it tends to persist. One year persistence of visual hallucinations and delusions in DLB has been reported to be 77% and 40% respectively (Ballard, O'Brien et al. 2001). Lower estimates of persistence over one year exist in the AD literature with Ballard et al. (2001) putting the figure at 26% and 44% for

hallucinations and delusions respectively. However, this is still a significant proportion of people and in other studies the estimates have been much higher, up to around 80% for one year down to 40-50% for both delusions and hallucinations in an eight year retrospective estimate (Craig, Mirakhur et al. 2005, Levy, Cummings et al. 1996).

Along with incidence and prevalence estimates, knowledge of risk factors will also help to refine the psychotic symptom phenotypes. Ropacki and Jeste (2005) conducted a large review of 55 studies to examine risk factors for psychosis in AD. The most notable risk factors (where over half the studies reported a significant association) were ethnicity, duration of illness and cognitive impairment. Cognitive impairment is of particular relevance as it is necessarily present in all studies of psychosis in dementia and is therefore discussed in more detail in the next section. There has not been such a review in DLB/PDD, however most studies support a relationship between more severe cognitive impairment and psychotic symptoms (see next section). The presence of early visuospatial deficits also has been identified as a predictor of hallucinations in DLB (Hamilton, Landy et al. 2011).

1.2.1 Relationship between cognitive impairment and psychosis in dementia

A key clinical feature associated with psychotic symptoms in AD is disease severity. The wide body of evidence supporting an association with ante-mortem measurements is further strengthened by post-mortem studies showing a correlation with severity of tau pathology, a key marker of disease severity (see section 1.4.3). Estimates of frequencies of hallucinations in mild, moderate and severe AD range from 0-21%, 10-23% and 22-56% respectively and there is a similar trend for delusions: 7-25%, 25-45% and 28-54% respectively (Lopez, Becker et al. 2003, Craig, Mirakhur et al. 2005, Piccininni, Di Carlo et al. 2005, Selbæk, Kirkevold et al. 2007). A five year follow up study plotting cognitive impairment quantitatively against symptom frequency found that by year 1 (when Mini Mental State Examination (MMSE) score was approximately 16, indicating moderate dementia) hallucinations and delusions increased from 8 to 16% and from 40 to 48% respectively (Holtzer, Tang et al. 2003). By year 5 (when MMSE was approximately 10, indicating more severe disease) the frequency of hallucinations declined to 13% while delusions declined to 34%, suggesting a non-linear relationship. Measures of quantitative symptom scores

rather than frequencies yield similar results, that is: hallucinations and delusions peaking in the moderate/moderate-severe stages (Suh, Kim 2004, Harwood, Barker et al. 2000).

The relationship between cognitive impairment and psychosis in DLB/PDD is not so clear, although there have been a number of studies investigating this link. In general most previous research supports a link with delusions or hallucinations in both PDD and DLB, with a slightly greater number supporting a link between hallucinations rather than delusions (Ballard, Holmes et al. 1999, Borroni, Agosti et al. 2008, Aarsland, Bronnick et al. 2007, Del Ser, McKeith et al. 2000, Aarsland, Cummings et al. 2001, Bronnick, Emre et al. 2011).

In addition to cognitive impairment, psychotic symptoms are also associated with faster rate of cognitive decline. McShane et al. (1997) found the severity of delusions (persecutory delusions specifically) to be associated with a 1.3-fold greater rate of cognitive decline (McShane, Keene et al. 1997). Drevets et al. (1989) found that over five years the performance of psychotic participants declined 11 points on the Blessed Dementia Rating Scale while non-psychotic individuals declined only 5 (Drevets, Rubin 1989). Interestingly, the rate of decline before the onset of psychotic symptoms has also been found to be a significant predictor of their appearance. In one study, every 1 point decline in the Mattis Dementia Rating Scale between baseline and the year prior to the development of psychotic symptoms was associated with a nearly 2-fold increase in risk for developing psychosis. Decline over the same period in verbal fluency was associated with a nearly 3-fold increase in risk for psychosis (Paulsen, Salmon et al. 2000). This latter finding may indicate that specific cognitive deficits such as executive function, or others that are implicated in frontal lobe function, may be of particular relevance to psychosis in dementia and some of the genes implicated in its aetiology (see section 1.4).

The evidence so far indicates that while delusions and hallucinations are certainly present in all stages of DLB/PDD there is a less strong link with disease severity than in AD, where the evidence is more robust. Here, there is a clear link between the increasing prevalence of psychotic symptoms and cognitive impairment. This has clear implications for the genetic studies of psychosis, given the substantial variation in cognitive impairment both within and between previous samples, and the impact of this is discussed in detail in section 1.4.7.1.

1.2.2 Heritability and familial aggregation of psychosis in Alzheimer's disease

Psychosis has been found to aggregate in siblings with AD and this has been influential in the search for genetic correlates. In the first study of its kind, Sweet et al. (2002) calculated that a sibling of an AD-psychosis proband had a 2.4-fold increased risk of psychosis. This figure refers to individuals who experienced psychosis at any point during their assessment period. Of significance, when the analysis was restricted to individuals who experienced only multiple symptoms (at one or more assessment) or recurrent symptoms (single or multiple) the risk increased to 3.8 (Sweet, Nimgaonkar et al. 2002). It should be noted that an unusually high number of participants were classified as psychotic, 75%. However two subsequent independent studies support these findings. In the first, treating only individuals with multiple symptoms (at least two delusion symptoms and at least one hallucination symptom), psychosis prevalence was 60% and the increased risk for psychosis in siblings of AD probands was nearly 4-fold (Hollingworth, Hamshere et al. 2007). In the second, the psychosis prevalence was 50%, with 36% experiencing multiple symptoms, and the risk associated with single and multiple/recurrent psychosis in the siblings of AD-psychosis probands was 2- and 3.8-fold respectively (Sweet, Bennett et al. 2010).

There has been less of a focus on the heritability of psychotic symptoms in AD, although at least one study has reported an effect consistent with the results above. Here, there was a suggestion of a stronger genetic component in multiple or recurrent psychotic symptoms, the authors calculated 30% heritability for a single psychotic symptom and 61% for multiple or recurrent symptoms (Bacanu, Devlin et al. 2005).

Heritability and familiality are not definitive proof of genetic causation. Although it is somewhat unlikely that elderly siblings share similar environments the possibility that certain early life experiences shared between siblings contribute to psychosis risk in AD cannot be excluded. However, that persistent symptoms may have a stronger genetic basis than non-persistent symptoms is a logical assumption. Psychotic-like phenomena, particularly hallucinatory symptoms, can arise out of acute episodes of delirium secondary to other medical conditions such as chest infections, stress and hospitalisation. The prevalence of delirium in dementia patients varies but ranges from 7%-22% in nursing home and community based samples to 80% in

hospital samples, with most cases resolving in around six months from onset (Fick, Agostini et al. 2002, Cole, McCusker et al. 2012). Along with the apparent high heritability and familial aggregation of recurrent symptoms this serves to highlight the importance of prospective evaluation of symptoms, so that causes of psychotic-like symptoms other than dementia itself can be excluded. This may even go some way to explain the lower heritability and familial aggregation in individuals with a single occurrence of symptoms in previous studies.

1.2.3 Treatment of psychotic symptoms in dementia

1.2.3.1 DLB/PDD

Consistent with the importance of cholinergic deficits highlighted in autopsy studies (Ballard, Piggott et al. 2000), there is randomised controlled trial (RCT) evidence of a modest overall improvement of neuropsychiatric symptoms, particularly visual hallucinations, with the cholinesterase inhibitor rivastigmine in patients with DLB and PDD (McKeith, Del Ser et al. 2000, Emre, Aarsland et al. 2004, Edwards, Royall et al. 2007). Although important, the overall level of improvement is modest and many individuals do not experience clinically significant improvements or they have significant residual psychotic symptoms. Most other trials in DLB and PDD have focused on antipsychotics.

Preliminary open trials of atypical antipsychotics in PD and DLB suggested some benefit in the treatment of neuropsychiatric symptoms (Onor, Saina et al. 2006, Fernandez, Trieschmann et al. 2002, Takahashi, Yoshida et al. 2003). There have been two RCTs of clozapine and one of olanzapine, all of which indicate a significant benefit (The French Clozapine Parkinson Study Group 1999, The Parkinson Study Group 1999) while RCTs of quetiapine and risperidone have shown no effect (Culo, Mulsant et al. 2010, Kurlan, Cummings et al. 2007). The risk of severe neuroleptic sensitivity reactions is substantial in these individuals (McKeith, Fairbairn et al. 1992) and safety issues, an FDA black box warning and the need for regular blood monitoring have limited their use.

1.2.3.2 Alzheimer's disease

The data currently available from DLB and PDD patients are not large enough or well characterised enough to warrant investigation of genetic determinants of treatment response, therefore the rest of this section will focus on treatments for AD. The lack of effective treatments in DLB and PDD does serve to highlight the importance of gaining a greater understanding of the aetiology of psychosis in these dementias, and genetic studies have an important role to play here.

To return to treatment options in AD, there have been a number of investigations of antidepressant efficacy for neuropsychiatric symptoms in general (i.e. not specifically psychotic symptoms). However, a recent meta-analysis only found evidence to support a modest benefit of selective serotonin reuptake inhibitor (SSRI) treatment when compared to placebo (Seitz, Adunuri et al. 2011). Importantly, the only one of the studies reviewed that examined psychotic symptoms specifically failed to find any evidence that they were more effective than risperidone, the only current licensed antipsychotic for psychosis in dementia (Pollock, Mulsant et al. 2007).

Two treatments for psychosis in AD deserve particular attention: antipsychotics and memantine. Antipsychotics are still widely used, despite the increased mortality rate associated with them, and it would be of benefit to understand the mechanisms underlying their harmful effects, particularly as alternatives are sought to replace their use. Memantine is a safe and well tolerated drug in AD however evidence of its efficacy in treating psychotic symptoms is mixed. Again, elucidating the genetic correlates of its efficacy may help identify poor responders or shed more light on how this drug exerts its effects; there has been no research in this area to date.

1.2.3.2.1 Antipsychotics in AD

Antipsychotics are a class of drug first developed for the treatment of schizophrenia but have long been the mainstay of pharmacological treatment of psychosis in AD. As a result there is a large body of literature evaluating their efficacy in this regard. Currently they are classified according to their affinity for blocking dopamine (DA) D2 receptors and the serotonin (5-hydroxytryptamine, 5-HT) receptor 2A ($5HT_{2A}$). The older agents, typical antipsychotics, have no affinity for $5HT_{2A}$ and the primary mechanism for their antipsychotic action is thought to be blockade of D2 receptors. Later hypotheses of psychosis incorporated the serotonergic system, particularly the $5HT_{2A}$

receptors, and as such a newer class of antipsychotic was developed targeting this receptor: the atypical antipsychotics. Many of these still have some affinity for D2 blockade but what characterises them pharmacologically is their much higher relative affinity for $5HT_{2A}$ (Meltzer, Matsubara et al. 1989).

Current evidence suggests risperidone may be efficacious in the treatment of psychosis in AD, and the current guidance reflects this as it is the only licensed treatment available. However, the results of a meta-analysis indicate that the benefit is likely to be only modest, with a weighted mean difference of -0.14 in favour of risperidone treatment over placebo (Ballard, Howard 2006). Despite risperidone being the only licensed treatment for psychosis in AD, other antipsychotics are still widely used, most notably among the atypical agents are quetiapine and olanzapine while haloperidol is the only typical agent still commonly used (Barnes, Banerjee et al. 2012). Similar (i.e. modest) effect sizes are found for aripiprazole and olanzapine as for risperidone but these are based on fewer trials, while there is very little evidence that quetiapine is effective (Ballard, Howard 2006, De Deyn, Drenth et al. 2013). There is also increased tolerability and mortality issues with haloperidol treatment (see below). In spite of their continued use, there are significant safety concerns surrounding the use of antipsychotics in people with AD, including risperidone.

Several studies have highlighted an increased mortality risk among patients with AD taking antipsychotics compared to those not. In a large meta-analysis of 15 RCTs totalling over 5000 participants Schneider et al. (2006) computed an overall 1.5-fold increased risk of death among individuals prescribed atypical antipsychotics. Individual analysis by drug showed a trend towards increased mortality in three of the drugs investigated (risperidone, olanzapine and quetiapine) while the significant increased risk persisted with aripiprazole (Schneider, Dagerman et al. 2006). A secondary analysis comparing haloperidol to risperidone and quetiapine generated a relative risk of 2, indicating that it is more harmful. Consistent with this evidence a large retrospective analysis by Wang et al. (2005) reported the use of typical antipsychotics to be associated with a 1.4-fold increased risk compared to atypical antipsychotics (Wang, Schneeweiss et al. 2005).

These two studies were followed by the DART-AD trial which followed up individuals five years after their participation in a clinical trial where their antipsychotic was either replaced with placebo or continued. Overall, the antipsychotic group had a significantly higher mortality rate, the

difference becoming most notable after 12 months and particularly so at 36 months where there was 30% survival in the antipsychotic group, compared to 60% in the placebo group (Ballard, Hanney et al. 2009).

There is not yet a mechanistic explanation for the harmful effects of antipsychotics, but one working hypothesis is that the sedating effects from histamine H1 antagonism cause secondary problems such as pneumonia and oedema (Ballard, Howard 2006). This hypothesis will be explored in more detail in section 1.4.6.1.

There is emerging evidence that pimavanserin, a newly developed $5HT_{2A}$ inverse agonist (Meltzer, Mills et al. 2010), is efficacious in the treatment of psychosis in the context of PD. The results of a subsequent six week trial in 199 patients released this year confirmed the preliminary 2010 results and trials in AD and other dementias are awaited (Cummings, Isaacson et al. 2013).

1.2.3.2.2 Memantine in AD

Numerous RCTs have shown memantine to be a safe and well tolerated drug in people with AD (Gauthier, Loft et al. 2008, Wilcock, Ballard et al. 2008, Fox, Breitner et al. 2012). It has also shown some efficacy in the treatment of psychotic symptoms.

In a pooled analysis of six clinical trials, Gauthier et al. (2008) found a significant effect of memantine (compared to placebo) in the treatment of delusions and hallucinations, as measured on the individual Neuropsychiatric Inventory (NPI) item sub-scales, over 12 weeks. Closer examination of this effect shows that symptoms actually remained more or less the same in the memantine group, while the placebo group worsened by 0.3 points on the NPI, against a baseline score of 3.9. The results at 24 weeks were similar although the effect of memantine on hallucinations was lost. Of patients symptomatic for delusions at baseline 62% improved after 24 weeks of memantine while 52% improved in the placebo group. No effect was found for hallucinations, although the magnitude of the difference was the same as that observed for delusions.

A more recent 12 week RCT failed to find any major effect of memantine on psychotic symptoms, with the only significant finding being an effect at six weeks when analysing an NPI cluster consisting of the delusions, hallucinations and agitation/aggression subscales (Fox, Breitner et al. 2012). Given previous data showing quite a strong effect of memantine on agitation and aggression (Wilcock, Ballard et al. 2008), it is plausible that this result was driven at least in part by these symptoms.

Memantine has two actions which are of potential interest in the treatment of psychosis in dementia; $5HT_{3A}$ and NMDA receptor (NMDAR) blockade.

In support of the hypothesis that $5HT_{3A}$ may be a relevant target of memantine, there is RCT evidence that ondansetron, a $5HT_{3A}$ antagonist, in combination with haloperidol is more effective than haloperidol alone for negative symptoms and psychopathology in schizophrenia (Zhang, Kang et al. 2006). Furthermore, in an early study ondansetron monotherapy was shown to be effective in the treatment of Parkinson's disease psychosis (Zoldan, Friedberg et al. 1995).

There has however been no research into $5HT_{3A}$ receptor changes and psychosis in dementia and much more attention has been paid to memantine's principal mechanism of action: NMDAR blockade. Abnormal glutamatergic stimulation of NMDARs is hypothesised to be a major pathway of neurodegeneration, and so their blockade by memantine may be protective against glutamate excitotoxicity (Danysz, Parsons et al. 2000). This is therefore thought to be central to a mechanistic explanation of its efficacy in treating AD itself. There is preliminary evidence from animal studies that memantine may inhibit and even reverse tau phosphorylation via PP2A (a major phosphatase implicated in abnormal tau phosphorylation in AD), while studies in humans have reported a reduction in CSF phospho-tau after one year of memantine treatment (Degerman Gunnarsson, Kilander et al. 2007). This is very early stage research and the exact relationship between NMDAR blockade and inhibition of tau phosphorylation is still not clear, however that tau is implicated in memantine's mechanism of action is of particular relevance to psychosis in AD given the association between NFT burden and psychosis (see section 1.4.3).

1.3 Section summary

AD, DLB and PDD together account for more than 70% of dementia cases. They are characterised by different patterns of progression clinically. Pathologically, the presence of NFTs and amyloid plaques distinguishes AD from DLB and PDD where Lewy bodies constitute the main hallmark pathological feature. Distinguishing between DLB and PDD, either clinically or pathologically is difficult and most experts support their grouping together for research purposes.

Psychotic symptoms (delusions and hallucinations) are common in DLB/PDD and AD. They are of central importance to each type of dementia because of their negative impact on disease course and the paucity of effective treatments which currently exist.

While well tolerated, cholinesterase inhibitors only appear to be of modest benefit in the treatment of visual hallucinations, and not effective at all in a large proportion of individuals with DLB and PDD. With regard to antipsychotics, the best evidence base for the treatment of psychotic symptoms in DLB/PDD appears to be clozapine, but tolerability issues and an FDA black box warning limit its use.

The picture is similar in AD. There is some modest evidence from meta-analyses that memantine may, in addition to being effective in the treatment of cognitive and functional decline, confer some benefit to the treatment of psychotic symptoms. However, more recent clinical trials have been disappointing. Antipsychotics have repeatedly been associated with increased mortality and a number of other side effects including sedation, oedema and pneumonia, and as a consequence guidance on their use in AD is restricted to short-term risperidone prescription, which itself appears to be only modestly effective.

There is an urgent need to identify safer and more effective therapies for psychosis in people with AD and DLB/PDD.

The first step in addressing this need has to be a greater understanding of the mechanisms underlying psychosis in dementia and in the absence of animal models genetic studies have a pivotal role to play in elucidating these. Moreover, genetic studies also have the potential to identify subgroups of likely poor responders, raising the possibility of more targeted therapeutic approaches using existing drugs.

1.4 Genetics of delusions and hallucinations in dementia and their treatment

The high heritability of psychotic symptoms in AD has led to numerous genetic association studies examining the relationship between polymorphisms in key psychiatric-related genes and the prevalence and severity of psychotic symptoms. The vast majority of these have been undertaken in AD (DeMichele-Sweet, Sweet 2010). Although a small number have also been carried out in DLB/PDD psychosis, research in this area is severely lacking (Kiferle, Ceravolo et al. 2007, Borroni, Di Luca et al. 2006). Genetic association studies have an important role to play in advancing current understanding of psychotic symptoms in dementia. Assessment of psychotic symptoms almost exclusively relies on self-report and in the case of dementia, most often requires the input of a care giver in order to rate them. They are therefore very difficult to model effectively in animals, with most models relying on appropriate observable correlates such as social withdrawal or cognitive dysfunction (Nestler, Hyman 2010). The issue is further complicated by the presence of dementia, its interaction with psychosis and the different clinical characteristics of psychosis in dementia compared with other neuropsychiatric illnesses on which current animal models are focused.

Post-mortem studies have undoubtedly played a key role in advancing understanding of psychosis in dementia, in particular because they allow researchers to localise neurobiological correlates. However one key drawback is that conclusions about brain changes and their clinical correlates are limited to the stage of dementia in which the donor died. Genetic association studies can provide important insights into the mechanisms underlying delusions and hallucinations, which are particularly difficult to investigate via the methods just described. This last point notwithstanding it is important to use data from the aforementioned techniques in order to generate hypotheses and select candidate genes.

A second potential benefit from the genetic association study method, which cannot be conferred from post-mortem studies, is in the prediction of treatment response, so called pharmacogenetics. This field is particularly attractive with respect to dementia given the current lack of effective

treatment strategies in this area and in particular the aforementioned adverse effects associated with antipsychotics. Pharmacogenetic research into treatments for psychosis in dementia has only started relatively recently, with the focus entirely on AD.

In the following sections four polymorphisms are discussed with respect to psychotic symptom prevalence and course in AD and DLB/PDD, antipsychotic mortality in AD and memantine efficacy in AD.

1.4.1 Serotonin transporter polymorphism and serotonergic correlates of psychosis in dementia

In the AD literature there have been a number of reported associations between polymorphisms in serotonergic genes and psychosis. The two most widely studied serotonergic polymorphisms are the $5HT_{2A}$ T102C single nucleotide polymorphism (SNP) and the serotonin transporter polymorphism, 5HTTLPR. In fact, all of the 15 serotonergic polymorphism studies in AD psychosis have examined one or both of these. Investigations of the $5HT_{2A}$ SNP were initially born out of the key role of this receptor in the psychosis inducing actions of psychoactive drugs such as LSD and the antipsychotic properties of atypical antipsychotics which exhibit some efficacy in ameliorating psychosis in schizophrenia (Beasley, Tollefson et al. 1997, Holmes, Arranz et al. 1998). It is a synonymous SNP and while this may mean it exerts its effects through influencing transcription or by being in linkage disequilibrium with the true causal variant, its functional consequences, if any, remain unknown.

5HTTLPR is a 44 bp deletion polymorphism in the promoter region of the serotonin transporter (5HTT) gene, *SLC6A4,* with two alleles; one termed long (L) and one short (S). *In vitro* studies have shown the S allele to be associated with a 3-fold lower rate of 5HTT transcription than the L allele as shown in Figure 1-2 (Lesch, Bengel et al. 1996, Collier, Stober et al. 1996, Heils, Teufel et al. 1996).

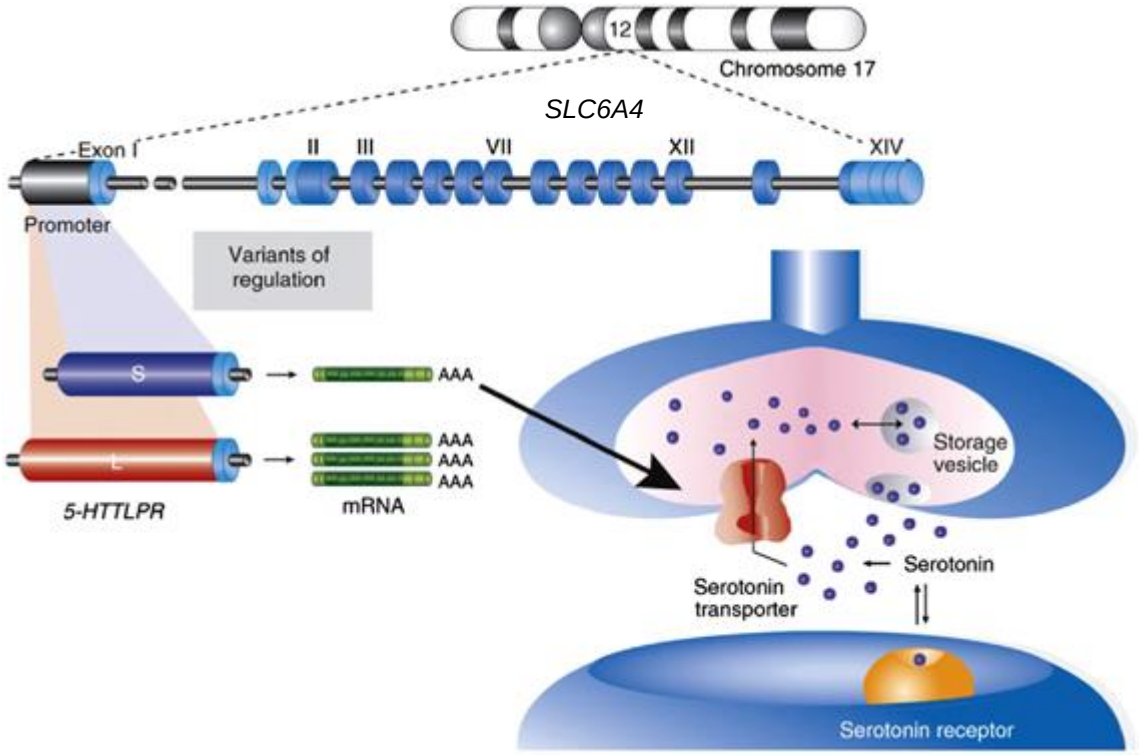

Figure 1-2 5HTTLPR polymorphism

A 44 bp deletion in the promoter of the SLC6A4 gene termed the short (S) allele results in a 3-fold lower rate of transcription than the long (L) allele, producing less mRNA and protein allowing less serotonin to be taken up into the presynaptic cell. Figure adapted from (De Neve 2011)

The serotonin transporter is expressed mainly in the 5HT synthesising neurons of the midbrain, in particular the raphe nuclei, with lower concentrations also found in some areas containing ascending 5HT projections like the frontal cortex and hippocampus (Lesch, Aulakh et al. 1993). Therefore, one hypothesis for a mechanistic explanation for the role of 5HTTLPR in psychosis in dementia is that the reduced transcription of the S allele leads to reduced protein levels causing less 5HT reuptake capacity with consequences for 5HT neurotransmission.

Post-mortem and PET studies, which have exclusively focused on healthy adults or adult neuropsychiatric syndromes other than dementia, have generated conflicting findings surrounding this hypothesis. In healthy adults, there is no clear evidence of an association between 5HTT availability and 5HTTLPR genotype with several *in vivo* imaging studies failing to find an association (Willeit, Stastny et al. 2001, Shioe, Ichimiya et al. 2003, van Dyck 2004, Jacobsen 2000, Murthy, Selvaraj et al. 2010). However another study, larger than those aforementioned (N=96), did report greater 5HTT availability in SS and LL genotypes compared to heterozygotes

(although only the SS/LS comparison reached statistical significance) (van Dyck 2004). This finding is difficult to interpret as one would not necessarily expect both homozygous groups to be similar. This finding may be a false positive, reflect differences in the populations studied or reflect the complexity of this particular polymorphism.

Evidence that the 5HTTLPR polymorphism may exert a different influence according to disease or disorder, and certainly evidence of the complexity of investigating these relationships, is provided by studies examining its effect in samples of adults with neuropsychiatric disorders other than dementia. Heinz et al. (2000), examining a dominant inheritance model of 5HTTLPR (i.e. LL vs. LS/SS), found non-alcoholic LL homozygous controls to have a significantly higher number of 5HTT sites in the dorsal raphe nucleus (DRN) than LL homozygous alcoholics (Heinz, Jones et al. 2000). Also examining a dominant inheritance model, Little et al. (1998) found that LS/SS carriers who were ethanol users had a significantly greater number of 5HTT sites than LS/SS carriers who were not ethanol users, again in the DRN, while binding potentials for LL carriers were similar (Little, McLaughlin et al. 1998). Interestingly, in the study by Little et al., there was some evidence that 5HTT binding may differ by brain region and by disorder. Specifically, cocaine users had higher 5HTT binding than ethanol users in the DRN but not in the median raphe (MRN) or substantia nigra (SN). The S allele has also been associated with lower 5HTT binding in suicide studies (Bah, Lindström et al. 2008, Du, Faludi et al. 1999), in particular in the frontal cortex, though it is important to note that contradictory evidence has also been presented (Mann, Huang et al. 2000, Parsey, Hastings et al. 2006).

The downstream effects of the 5HTTLPR polymorphism in terms of 5HTT binding density are clearly complex, but it is sensible to hypothesise that any effects in binding may be disease specific and brain region specific. In addition, there is evidence that the influence of 5HTTLPR may also extend to affect 5HT receptors which are implicated in a variety of psychiatric disorders and thus may be important for psychosis in dementia, namely $5HT_{2A}$ and $5HT_{1A}$.

Alterations in density of both of these receptors have been observed in 5HTT knockout mice, highlighting a potentially important relationship in humans. $5HT_{1A}$ receptor density is significantly reduced, particularly in the DRN, MRN, hypothalamus and amygdale of 5HTT -/- mice, while there were no changes in the frontal cortex or hippocampus (Li, Wichems et al. 2000). $5HT_{2A}$ receptors

on the other hand are affected to a much lesser extent in 5HTT knockouts, with density increased in the hypothalamus and decreased in the striatum (Li, Wichems et al. 2003).

The findings above regarding $5HT_{1A}$ are supported by PET imaging in humans which has demonstrated the SS genotype to be associated with lower $5HT_{1A}$ binding potential than the LL genotype (David, Murthy et al. 2005). However, two later studies reported greater binding potential associated with the SS genotype and another found no association at all (Lothe, Boni et al. 2009, Borg, Henningsson et al. 2009, Lee, Bailer et al. 2005). The reasons for these conflicting findings are not clear, however in common to all of these studies no impact has been found on $5HT_{1A}$ autoreceptors in the brainstem nuclei from where 5HT neurons originate (DRN and MRN).

It is well established that 5HTTLPR affects *SLC6A4* transcription, with the SS genotype being associated with a significantly reduced rate of transcription compared with the LL genotype. What is not so clear however is how this might translate into a mechanism for psychiatric symptoms in dementia. It is possible that the 5HTT reuptake sites are affected, with there being some evidence for this in depression and substance abuse. Evidence from 5HTT knockout mice, supported by preliminary PET imaging in man, points to a possible role for 5HTTLPR in altering $5HT_{1A}$ receptor density in many cortical areas of the brain. Like the 5HTT binding studies, the direction of this relationship appears to be region specific and it is not yet known whether it too is disease/disorder specific.

To date there have been no biochemical studies investigating the above links in dementia. However post-mortem studies, although there are not many focusing specifically on psychotic symptoms, have highlighted the serotonergic system more generally as an important correlate in AD and DLB/PDD.

With regard to AD, a significant decrease in 5HT concentration and increase in acetylcholinesterase (AChE)/5HT ratio in the ventral temporal cortex (BA20), but not the anterior prefrontal cortex (BA10), has been shown to predict psychotic symptoms (Garcia-Alloza, Gil-Bea et al. 2005). 5HTTLPR may fit into this hypothesised mechanism by compounding 5HT levels reduced through neurodegeneration, in this instance it would be the LL genotype that would

increase susceptibility to psychotic symptoms relative to SS carriers. The only other study specifically to address the relationship between serotonergic system changes and psychotic symptoms in AD focused on hippocampal regions and found no evidence to suggest that $5HT_{1A}$, $5HT_{2A}$ or 5HTT density is associated with these symptoms in this region (Lai, Tsang et al. 2010).

Cheng et al. (1991) reported preserved $5HT_2$ receptor (though they did not discriminate between $5HT_2$ subtypes) binding in the temporal cortex of DLB patients with hallucinations compared to those without (Cheng, Ferrier et al. 1991). This evidence is supported by Perry et al. (1990) who cite a monoaminergic-cholinergic imbalance as an important neurochemical correlate (Perry, Marshall et al. 1990). Specifically, the ratio of 5HTIAA (the main metabolite of 5HT) to choline acetyltransferase (ChAT) was 9.3 in DLB patients with hallucinations compared to 3.1 in those without hallucinations (which was comparable to control). Again this analysis focused on the temporal cortex.

It is noteworthy that the direction of the ratio associated with hallucinations in DLB (Perry, Marshall et al. 1990) is similar to that reported by Garcia-Alloza et al. (2005) in AD (although different markers were used). Establishing common and distinguishing mechanisms underpinning the same symptoms across the AD/DLB/PDD spectrum will be important in advancing current understanding of these symptoms and implementing new treatment strategies.

There is a lack of research addressing the relationship between serotonin dysfunction and psychotic symptoms in PDD but in PD with psychosis a recent PET study reported findings similar to Cheng et al. (1991) (Ballanger, Strafella et al. 2010). The authors extended the analysis to examine frontal cortical areas as well as temporal cortical areas and reported increased $5HT_{2A}$ binding in several regions in each to be associated with hallucinations. Moreover, in a post-mortem study of PD psychosis, Huot et al. (2010) have presented findings again supporting a role for the relative preservation of $5HT_{2A}$ receptors in the temporal cortex (specifically BA21) in PD patients with hallucinations compared to those without hallucinations. In contrast to Ballanger et al, Huot et al found no evidence of binding differences in the orbitofrontal cortex according to the presence of hallucinations (Huot, Johnston et al. 2010).

Although the patterns of association with changes in serotonergic neurochemistry and psychotic symptoms still require more research, there is evidence that this is an important correlate in AD and DLB. There is even evidence to suggest that similar patterns of dysfunction underpin psychotic symptoms in these two dementias. Although there have been no studies specifically focusing on PDD as it is very closely related to PD and DLB it might be expected that similar changes would be observed here.

Taken together the above evidence strongly implicates brain region-specific serotonergic alterations in the aetiology of psychotic symptoms in dementia. Although more post-mortem and PET research is needed, functionally significant polymorphisms such as 5HTTLPR provide a good opportunity to complement and enhance the findings from neurochemical research.

There have been ten studies to date which have investigated the role of the 5HTTLPR polymorphism and psychotic symptoms in AD (Proitsi, Lupton et al. 2010, Borroni, Grassi et al. 2006, Borroni, Grassi et al. 2006, Albani, Prato et al. 2009, Pritchard, Pritchard et al. 2007, Quaranta, Bizzarro et al. 2009, Sweet, Pollock et al. 2001, Rocchi, Micheli et al. 2003, Ueki, Ueno et al. 2007, Ha, Cho et al. 2005). Of these, two were carried out in very similar cohorts but with a slightly different approach to classifying psychosis (Borroni, Grassi et al. 2006, Borroni, Grassi et al. 2006). This section will review the results of these studies and their implications but section 1.4.7 will discuss in detail how the methodological and analytical differences in these studies may have contributed to the differences in findings.

Excluding Borroni et al. (2006b), the study which took the more different approach to analysis (i.e. factor analysis instead of examining symptom frequencies by genotype) and whose cohort was probably largely overlapping with Borroni et al. (2006a), four out of nine previous studies on 5HTTLPR have reported an association with psychotic symptoms in AD. However, the direction of the relationship is not consistent between these four studies. Both Sweet et al. (2005) and Quaranta et al. (2009) reported a positive association between the L allele and LL genotype and psychotic symptoms with odds ratios (ORs) for the LL genotype of 2.6 and 7.25 for each study respectively. Conversely the study by Borroni et al. (2006) reported the S allele (OR: 2.14) to be the risk factor, also interacting with the *COMT* high activity val allele to further increase the OR to 9.6 (see section 1.4.2 for explanations of the *COMT* val158met polymorphism). Lastly, no main

effect of 5HTTLPR genotype was found by Proitsi et al. (2010), there was just an interaction between the SS genotype and absence of the *COMT* high activity val allele.

The findings in AD are suggestive of an association between 5HTTLPR and psychotic symptoms. Further investigation is warranted, in light of the methodological concerns outlined later but also because, as described previously, the functional consequences of both the S and L allele can plausibly fit mechanisms of psychosis. It is also discussed in section 1.4.7 how this polymorphism may fit into more complex explanations of psychosis in dementia.

There are few studies examining 5HTTLPR in neurodegenerative disease psychosis other than in the context of AD. In one that is of particular relevance, Kiferle et al. (2007) examined the relationship between 5HTTLPR and visual hallucinations in PD and found no association (Kiferle, Ceravolo et al. 2007). There have to date been no studies of the 5HTTLPR polymorphism in DLB/PDD so there is a need to expand genetic association studies to include this. Given the paucity of studies in these two diseases it is at present only possible to begin to draw inferences from AD, and caution must still be exercised here.

1.4.1.1 Section summary

The 5HTTLPR polymorphism results in a 44 bp deletion in the promoter of the serotonin transporter gene, *SLC6A4*. The allele with the deletion (S) is associated with an approximately 3-fold lower rate of transcription than the long (L) allele, leading to less messenger RNA (mRNA) and functional consequences which are supported by animal studies and *in vivo* imaging in humans. Given the good body of evidence from post-mortem studies in humans linking serotonergic dysfunction with psychotic symptoms in DLB/PDD and AD, 5HTTLPR is a good candidate polymorphism and has the potential to contribute to a mechanistic explanation of these symptoms.

5HTTLPR is one of the most well studied polymorphisms in AD psychosis research. Of the nine independent studies carried out to date, four have reported a significant association, two with the LL genotype, one with the SS genotype and one interaction with the *COMT* val158met polymorphism. The inconsistent findings may be the result of a number of methodological

differences, which are discussed in more detail in section 1.4.7, however they do provide an indication that this polymorphism may be of potential importance to the pathogenesis of psychotic symptoms in AD. There is also an urgent need to extend this research in DLB/PDD, with the goal of identifying common mechanisms which underpin the same symptoms in different types of dementia.

1.4.2 Catechol-O-methyltransferase val158met polymorphism and dopaminergic correlates of psychosis in dementia

The catechol-O-methyl transferase (*COMT*) val158met polymorphism is another relatively well studied polymorphism in AD psychosis research, and is the only polymorphism to also have been investigated in DLB. Like the serotonergic gene research described above there has been a reasonable focus on other polymorphisms in dopaminergic genes, in particular those in DA receptors D1-4 (Sweet, Nimgaonkar et al. 1998, Holmes, Smith et al. 2001, Proitsi, Lupton et al. 2010, Craig, Hart et al. 2004, Sato, Ueki et al. 2009, Pritchard, Ratcliffe et al. 2009) and the DA transporter (DAT) (Pritchard, Pritchard et al. 2008, Proitsi, Lupton et al. 2010). Of all the dopaminergic polymorphisms examined in relation to psychosis in AD among the most convincing biochemical and molecular genetic evidence for functional impact on putative disease mechanisms comes from the *COMT* val158met polymorphism. The same rationale for focusing on 5HTTLPR, as opposed to $5HT_{2A}$, can be applied to *COMT* val158met in this instance. Specifically although there is evidence that the DAT polymorphism alters transcriptional efficiency (Miller, Madras 2002) there is little evidence of its functional significance to psychosis in AD beyond this.

On the other hand, *COMT* val158met has been extensively studied and there is solid evidence on which to base hypotheses concerning its role in the aetiology of psychotic symptoms in dementia.

COMT is an enzyme responsible for terminating the action of dopamine and is encoded by a single gene on chromosome 22. There are two COMT isoforms, the soluble 1.3kb transcript (S-COMT) and the longer 1.5kb membrane bound transcript (MB-COMT). MB-COMT (henceforth COMT) is the only one of the two that is expressed in the CNS (Hong, Shu-Leong et al. 1998). The *COMT* val158met polymorphism is a g/a SNP which leads to a valine (val)/methionine (met)

substitution in the amino acid sequence of the resulting protein (it is present in both isoforms), see Figure 1-3. The met allele is significantly less heat stable at body temperature and is therefore associated with a 2-3 fold lower enzyme activity. The presence of val, the so-called high activity allele, is associated with a downregulation of dopamine activity in the prefrontal cortex (PFC), which may in turn possibly increase expression of the DA precursor tyrosine hydroxylase in subcortical areas (Akil, Kolachana et al. 2003, Matsumoto, Weickert et al. 2003).

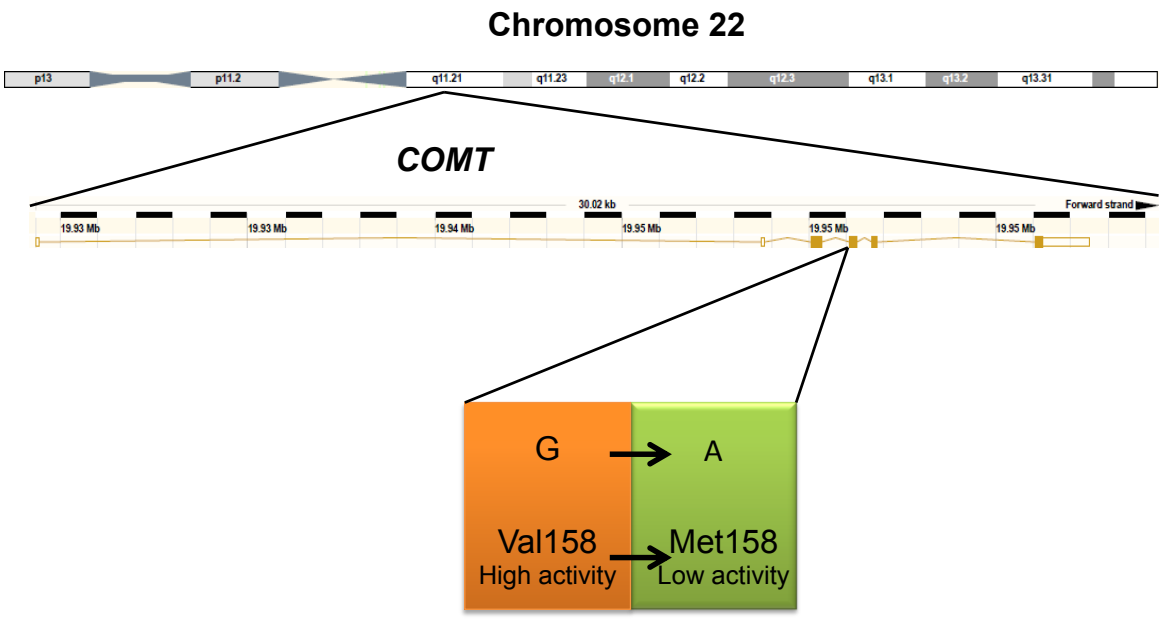

Figure 1-3 *COMT* val158met polymorphism

Contained in exon 4 of the COMT gene, the methionine coding allele is less thermostable than the valine allele, resulting in a lower activity enzyme associated with less efficient dopamine clearance and therefore higher dopamine levels. This is particularly relevant for prefrontal areas where COMT is the major route of dopamine inactivation

COMT is expressed widely in the human brain but it is its expression in the PFC that has garnered considerable attention in the field of psychiatry. In the human brain dopamine transporters are another major route of dopamine inactivation, however they are not abundantly expressed in the PFC (Matsumoto, Weickert et al. 2003). Furthermore *COMT* knockout mice show a 2- to 3-fold increase in dopamine levels in the dorsolateral prefrontal cortex (DLPFC) but not the striatum, providing further evidence that it is the major regulator of dopamine neurotransmission in this brain region (Gogos, Morgan et al. 1998).

The downstream effects of the changes in DA tone associated with *COMT* val158met have yet to be fully elucidated but a number of PET imaging and post-mortem studies have been carried out which provide some suggestions.

In human PET studies, involving healthy adults, the *COMT* high activity val allele has been associated with an increase in the number of cortical (including DLPFC) and limbic dopamine D1 receptors, consistent with a decrease in DA tone associated with this allele (Slifstein, Kolachana et al. 2008). A similar PET study however found no association between the *COMT* val158met polymorphism and striatal or cortical D2 or D3 receptor availability (Hirvonen, Någren et al. 2010). There are few studies of this kind (that is, examining changes in dopamine receptor availability) so it would be premature to draw concrete conclusions, however this pattern of receptor change, involving the DLPFC, is worth noting.

There is evidence implicating D2 receptor changes in the pathogenesis of psychotic symptoms in disorders other than dementia such as schizophrenia and bipolar disorder. Specifically, increased striatal D2/D3 receptors have been observed in bipolar patients with psychosis compared to those without (Pearlson 1995). In AD, a more recent PET study noted a similar pattern: a significant correlation between the delusion score on the NPI and dopamine D2 binding potential (Reeves, Brown et al. 2009), while a post-mortem study of AD cases found a selective increase of D3 receptors, but not D2 or D1 receptors, among those with psychosis (Sweet 2001). These studies in AD provide support for a hypothesis implicating dopaminergic neurotransmission in the aetiology of psychotic symptoms. However, to return to the *COMT* val158met polymorphism, although limited, PET evidence links the val allele to D1 receptor changes, not D2 or D3.

There is a much larger body of evidence implicating D1 receptors in cognition rather than psychosis in the wider literature, consistent with the dopamine hypothesis of schizophrenia which states that hypostimulation of PFC D1 receptors is responsible for negative and cognitive symptoms while hyperstimulation in subcortical areas is responsible for positive symptoms (Abi-Dargham 2004). Therefore an arguably more likely hypothesis with respect to psychosis in AD, and certainly one for which there is more evidence, is that the psychological phenotype associated with *COMT* val158met would be cognitive in nature, perhaps meditated via D1 receptors. In healthy individuals tested before and after cognitive training, larger decreases in D1

binding potential have been associated with greater improvements on working memory tasks (McNab, Varrone et al. 2009) while in schizophrenics higher D1 binding potential is associated with worse performance in cognitive tests (Abi-Dargham, Mawlawi et al. 2002). This relationship will be discussed in detail in section 1.4.7.2 however it is important to note at this stage that the influence of *COMT* val158met, and indeed 5HTTLPR, on psychotic symptoms may not be a direct one.

The relationship with D1 receptors notwithstanding, the above studies highlight the importance of dopaminergic neurotransmission in the aetiology of psychosis and also draw attention to the possibility that psychotic symptoms may share common neurobiological correlates regardless of the principal psychiatric diagnosis of the patient (Reeves, Brown et al. 2009, Pearlson 1995).

Taken together, the evidence surrounding DA receptor changes in psychotic symptoms in AD and the known physiological consequences of the *COMT* polymorphism presented above provides evidence that the *COMT* val158met polymorphism might be implicated in psychosis in dementia. However, the previous research in this area has yielded mixed results.

There have been seven previous investigations of the *COMT* val158met polymorphism and psychotic symptoms in AD. Four of these have been conducted by the same group in Italy (Borroni, Grassi et al. 2007, Borroni, Di Luca et al. 2006, Borroni, Grassi et al. 2006, Borroni, Grassi et al. 2006). All four of these studies found the high activity val allele to increase risk for psychosis. However, it would be prudent not to attribute any more weight to these findings than would be attributed to any one study, given that there is likely to be significant or complete overlap in patients included in each of the four studies (the authors were contacted to confirm this but no response was received). In Borroni et al. (2006b) a 2.4- and 2.7-fold increased risk of psychosis was associated with *COMT* val allele and val/val genotype respectively and as noted in section 1.4.1 an interaction was also found between *COMT* val and the 5HTTLPR S allele. In further support of the val allele carrying the risk, Sweet et al. (2005) found the risk of psychosis to be increased in individuals with each copy of the val allele carried (Sweet, Devlin et al. 2005). However, in the third independent study in AD, again as noted in section 1.4.1, Proitsi et al. (2010) did not find a main effect of the *COMT* polymorphism but they did find a significant interaction between the 5HTTLPR SS genotype and absence of the *COMT* val allele (Proitsi,

Lupton et al. 2010). Finally, in a small DLB study the val/val genotype was again found to raise risk for psychosis (Borroni, Di Luca et al. 2006), while in the only study to be carried out in PD psychosis, no association was found (Camicioli, Rajput et al. 2005).

1.4.2.1 Section summary

Compared to serotonin, there is less research surrounding dopaminergic neurotransmission and psychotic symptoms in AD. However the evidence that does exist, along with the previous positive associations of *COMT* val158met in AD psychosis studies suggests this polymorphism and by extension dopaminergic neurotransmission may be important in the aetiology of psychotic symptoms.

Although only two out of three previous studies have reported the val allele as a risk for psychosis this is indicative of lower dopaminergic neurotransmission, probably in the frontal cortical areas, being associated with psychosis in AD. With the same finding being reported in a preliminary DLB study, there is an indication that psychotic mechanisms are perhaps shared in the two disorders. Given what is known about *COMT* expression in the brain, it could be speculated that frontal cortical regions are important in this mechanism. However, it is important to note that these regions are also implicated in cognition which is itself a correlate of psychotic symptoms, providing a further reason to clarify the role of this polymorphism in well characterised cohorts.

1.4.3 Tau, the *MAPT* haplotype and psychosis in AD

In AD and other tauopathies the ratio of 4R to 3R tau is disrupted and this is thought to play a role in the neurodegenerative process, though the exact mechanism is not known. To focus on AD, post-mortem studies have shown that relatively less 3R tau is expressed in AD when compared to controls. In the nucleus basalis tau 4R expression was 1.4-fold greater than 3R expression in AD cases, a significant difference to the control ratio of 1:1 and a shift attributed to less expression of 3R as opposed to greater expression of 4R (Ginsberg, Che et al. 2006). An earlier study with more extensive brain region coverage reported similar findings, but notably the 4R:3R ratio was only significantly higher in AD compared to controls in those regions that are typically heavily burdened with NFTs in AD (namely, the hippocampus, medial frontal cortex and medial temporal

cortex, but not in the entorhinal cortex). The occipital and motor cortices, the caudate and cerebellum all showed a similar ratio to control brains (Yasojima, McGeer et al. 1999). Absolute levels of 4R tau were also significantly higher in AD compared to control in the heavy tau burden areas, while 3R tau expression was significantly lower. It is therefore important to note the complexity of tau isoform expression; not only does it depend on disease but it is also brain-region specific.

The above only accounts for tau isoform expression, rather than abnormal phosphorylation and aggregation into NFTs, but suggests that alterations in tau transcription may precipitate the formation of NFTs in AD. A comparison between NFT composition in AD and progressive supranuclear palsy (PSP) found that in the basal ganglia, another region heavily affected by tau pathology in AD, detergent insoluble tau fragments were composed of less 4R tau than 3R tau by a factor of around 0.75, while the reverse was found for patients with PSP (Liu, Le et al. 2001). This was examined in only one brain area but is consistent with NFTs in AD being composed of all *MAPT* isoforms.

The significance of tau in AD psychosis will be discussed in further detail below, but at this stage it is important to note that tau pathology is complex. It appears that disruption of the 4R:3R ratio, either in favour of relatively more of either isoform is associated with different tauopathies and that, in addition, in certain brain regions the 4R:3R ratio is more susceptible to being perturbed.

There is a body of literature linking tau pathology to psychosis in AD. Early post-mortem studies reported higher levels of NFT pathology to be associated with psychotic symptoms, although the exact patterns of association differ slightly. Zubenko et al. (1991) examined a psychosis syndrome (delusions and/or hallucinations) and tangle pathology in four brain regions: the medial frontal and superior temporal cortices, the entorhinal cortex (which connects the hippocampus with many other regions, notably the amygdala, cingulate and frontal cortex) and the presubiculum (an area with close connections to the hippocampus) (Zubenko, Moossy et al. 1991). The authors found that patients with psychosis had significantly higher densities of tangle pathology in the middle frontal cortex and presubiculum than those without. Subsequently, Forstl et al. (1994) examined separate psychotic symptoms, namely persecutory delusions, misidentifications, visual hallucinations and auditory hallucinations (Forstl, Burns et al. 1994). The

authors failed to replicate the earlier association found by Zubenko et al. of tangle density in the medial frontal cortex and psychosis. They did however, again unlike the earlier finding, report a 2-fold higher number of NFTs in the parahippocampus gyrus (which includes the entorhinal cortex) in patients with any type of delusion compared to those without. No association was found with any other type of psychotic symptom. Forstl et al.'s failure to replicate the previous findings of Zubenko et al. may well be a result of them beginning with the assumption that individual psychotic symptoms may differ in their aetiology, therefore highlighting the importance of treating individual symptoms separately, particularly when evaluating their role in disease mechanisms.

Two later studies again examined NFT pathology in some of the areas studied previously (namely CA1 and the entorhinal cortex of the hippocampus, the medial frontal cortex and the superior temporal gyrus) (Sweet, Hamilton et al. 2000, Farber, Rubin et al. 2000). Farber et al. found a 2- to 3-fold greater density of tangles in patients with psychosis (delusions and/or hallucinations) than those without in the medial frontal and superior temporal cortices but not the hippocampus or entorhinal cortex. That is, they found similar findings to Zubenko et al., but not Forstl et al., perhaps due to the similar measure of psychosis employed. Conversely, Sweet et al. failed to find any association between severity of tangle pathology and psychosis in the hippocampus, medial frontal or superior temporal cortices. There is however more emerging preliminary evidence that phospho-tau in the DLPFC is higher in AD patients with psychosis (Murray, Kirkwood et al. 2013) and other recent evidence suggesting that levels are also raised in agitation and aggression (Guadagna, Esiri et al. 2012). The exploration of the relationship between tau pathology and psychosis is made more complicated by the relationship between disease severity and both tau pathology and psychosis. Genetic association studies offer the valuable opportunity to explore this relationship over the disease course, having the potential to bring greater clarity to current understanding of this relationship.

One source of *MAPT* genetic variation that appears to affect the transcription of different tau isoforms is the extended *MAPT* haplotype. The extended *MAPT* haplotype exists in two forms, termed H1 and H2. The H2 allele exists exclusively in Caucasians: from a practical point of view H2 frequencies are 0% in Native American, East Asian and African populations (Evans, Fung et al. 2004). Moreover, a variant of the H1 clade, H1c, has been identified as important in itself,

possibly driving the associations previously attributed to H1 (Myers, Kaleem et al. 2005, Myers, Pittman et al. 2007).

The *MAPT* haplotype has been shown to have functional consequences. In control brains with no evident neuropathology the H1 haplotype is associated with a 1.3- to 1.4-fold increase in 4R tau expression relative to the H2 haplotype in the globus pallidus while the figure in the frontal cortex is slightly lower at 1.2- to 1.3-fold higher (Caffrey, Joachim et al. 2006). Subsequently the same group showed that the H2 haplotype is associated with a 2-fold increase in 2N tau expression relative to the H1 haplotype (see Figure 1-1 for a depiction of tau isoforms) (Caffrey, Joachim et al. 2008). These results do not allow any conclusions to be drawn about the propensity of isoforms resulting from either haplotype to be abnormally phosphorylated, but they are important in illustrating possible mechanistic effects of the haplotype. In AD, the tau H1 and the H1c haplotype have been implicated as a risk for the disease, but this has not been universally replicated (Myers, Pittman et al. 2007, Baker, Graff-Radford et al. 2000, Russ, Powell et al. 2001, Myers, Kaleem et al. 2005). Moreover, in a mixed AD and control cohort (analysed this way because no expression differences were found between cases and controls) the H1c haplotype has been associated with increased total tau expression and a relatively higher increase in 4R tau expression (25%) compared to other haplotypes (Myers, Pittman et al. 2007). The implication here is that the tau H1c haplotype is responsible for causing a greater proportion of 4R tau to be expressed and that this is in turn implicated in tangle formation. It is noteworthy that overall tau expression was not associated with the haplotype in the studies by Caffrey et al. (2006, 2008), in contrast to that of Myers et al. (2007). This perhaps reflects that the H1c hapolotype is the true driver of higher expression and that analysing the H1 haplotype only masks important differences. However, a more recent study in healthy adults suggests a more complex picture in which the H1 haplotype may have a distinct role. Hayesmoor et al. (2009) found no effect of H1c haplotype on total tau expression but they did report a strong negative correlation between H1 expression as a proportion of total tau expression (i.e. from H1 and H2 haplotypes) and age at death (Hayesmoore, Bray et al. 2009).

The complexity of this haplotype is further illustrated by evidence from the wider literature highlighting that the H2 haplotype may negatively impact disease, despite being generally found to be protective in most tauopathies. Specifically, the H2 haplotype has been associated with a

lower age of onset in both FTD and dementia associated with Down's syndrome (Jones, Margallo-Lana et al. 2008, Borroni, Yancopoulou et al. 2005, Laws, Perneczky et al. 2007). In this latter study by Laws et al. (2007) the H2 haplotype was also associated with more severe decline of glucose metabolism in the frontal cortex of FTD patients. This may be of relevance in AD psychosis because there is evidence suggesting the dysfunction of glucose utilisation in prefrontal brain regions is correlated with the presence of delusions (Sultzer, Brown et al. 2003, Mentis, Weinstein et al. 1995). This is not a universal finding however as the only other cohort study examining this relationship failed to find any difference in metabolism in frontal brain areas but did report hypometabolism and hypermetabolism in the medial occipital and inferior temporal cortices respectively (Hirono, Mori et al. 1998). Whether this effect is present and mediated by the tau H2 haplotype in AD remains to be established, but the current evidence could implicate it as a putative psychotic symptom risk factor.

There has been one genetic association study of the extended *MAPT* haplotype in AD psychosis research. DeMichele-Sweet et al. (2011) in one of the largest and most well characterised studies of its kind tagged the entire *MAPT* gene but did not find an association with psychosis (DeMichele-Sweet, Klei et al. 2011). Although this is evidence against *MAPT* genetic variation playing a role in psychosis, and in view of the study design and cohort can be considered reasonably robust, there are two important points to highlight. Firstly, the authors examined delusions and hallucinations together and as has been highlighted in several sections previously there is a good body of evidence that these symptoms do not completely share the same aetiology. Secondly, tau may not be associated with the presence or absence of psychosis; however it could act as a modifier once psychosis has set in causing it to become more severe. This would be analogous to a disease modifying hypothesis, for example the aforementioned study by Laws et al. (2007) found no association between the tau haplotype and FTD but did find it modified age at onset, suggesting that it could represent a risk for FTD with a different clinical presentation, once neurodegeneration as set in.

1.4.3.1 Section summary

In post mortem studies, a greater burden of NFT pathology has been associated with psychotic symptoms in AD with a good degree of consistency, suggesting AD with psychotic symptoms may

represent a form of the disease with a more severe pathology. Further exploration of this hypothesis is possible through a genetic association approach because of the advantage of being able to serially evaluate participants and correlate 'real time' clinical data with genetic variation which has known functional significance. One such source of variation is the extended *MAPT* haplotype which has been shown to affect total tau transcription and 4R isoform expression in a brain region specific manner with possible implications for NFT pathology and thus psychosis.

1.4.4 Other polymorphisms associated with psychosis in dementia

Besides the polymorphisms outlined above, there is a reasonable body of literature pertaining to genetic associations of other putative psychosis risk genes, comprising at least 34 studies in total. Of these, 22 examined the role of *APOE*, the single biggest genetic risk factor for sporadic AD, which have been identified in a review by DeMichele-Sweet et al (2010). Of these 22, eight found a significant association (DeMichele-Sweet, Sweet 2010). Two much larger subsequent studies (N~800) examined *APOE* again, one failed to find an association (Demichele-Sweet, Lopez et al. 2011), but the other did report an association (Christie, Shofer et al. 2012). What is particularly important about this latter finding is the phenotype that was examined. The authors examined two phenotypes, one with people who suffered both delusions and hallucinations, and one with delusions only. Hallucinations alone could not be examined as 94% of those with hallucinations also had delusions. Under the delusions and hallucinations phenotype the *APOE**4 allele emerged as protective against hallucinations (OR: 0.7 for one allele and 0.3 for two), while there was no effect observed when examining people with delusions but not hallucinations. Given the size of the study this represents strong evidence that the previous psychosis phenotype of delusions and/or hallucinations is not appropriate for genetic studies.

1.4.5 Genome-wide association and linkage studies in Alzheimer's disease psychosis

Genome-wide association studies (GWAS) are becoming increasingly common in AD as a whole and have yielded some influential findings (Hollingworth, Harold et al. 2011, Harold, Abraham et al. 2013). Such studies require large numbers of cases to generate sufficient power and this has been the biggest barrier to their use in AD psychosis research. One GWAS paper has been published, but no hits reached genome-wide significance. This study was probably

underpowered, however some sub-threshold loci were identified namely in the visinin like protein 1 (*VSNL1)* and solute carrier family 2, facilitated glucose transporter member 9 *(SLC2A9)* genes. Although not implicated in psychosis in AD previously, the associated proteins have been implicated in schizophrenia and AD.

Linkage studies are another method that is useful in establishing whether psychotic symptoms have a genetic basis. This method has been employed in three studies investigating AD psychosis (Avramopoulos, Fallin et al. 2005, Bacanu, Devlin et al. 2002, Hollingworth, Hamshere et al. 2007). Genome-wide significant linkage was found at loci on chromosome 14, 15 and 2 respectively and in two cases this indicated allele sharing among relatives without psychotic symptoms (Hollingworth, Hamshere et al. 2007, Avramopoulos, Fallin et al. 2005). The genetic linkage studies in this area have therefore not provided consistent evidence, although arguably the most plausible candidates to arise from this method are the nicotinic acetylcholine receptors located in the region identified on chromosome 15 by Hollingworth et al (2007).

1.4.6 Pharmacogenetics of treatments for psychosis in AD

Antipsychotic agents target a diverse range of receptor systems, including cholinergic, serotonergic, dopaminergic, noradrenergic and histaminergic receptors. Actions at many of these systems are key mediating pathways in the common adverse events associated with this class of drugs, and could potentially be influenced by genetic polymorphisms affecting these receptor systems. In addition, there is known genetic variability in the pathways responsible for the metabolism and breakdown of antipsychotic drugs, which could also impact on the severity of adverse events. Pharmacogenetics therefore offers an exciting opportunity to contribute towards effective individualised medicine, where genetic polymorphisms could inform our understanding of the severity of potential risks and likelihood of benefit of specific individuals to antipsychotic and other therapies, and possibly inform the optimal starting dose.

Despite the potential opportunities, this area has not been well studied in dementia. Of note however, one well-controlled proof of concept study (Dombrovski, Mulsant et al. 2010) highlighted that the 5HTTLPR SS genotype was associated with reduced treatment response and an increased propensity of adverse events during risperidone treatment. There have been two other

much smaller cohort studies examining antipsychotic treatment response in AD. Both focused on the $5HT_{2A}$ T102C polymorphism and examined antipsychotic efficacy, rather than modification of side effects or adverse reactions (Angelucci, Bernardini et al. 2009, Engelborghs, Holmes et al. 2004).

The study by (Dombrovski, Mulsant et al. 2010) is therefore the first indication that genetic variants in pathways targeted by commonly used psychotropic drugs may be useful in predicting response (with respect to adverse events) in individuals with dementia. An earlier study also provides a good indication that pharmacogenetics may inform the likelihood of adverse events in response to antipsychotic drugs in people with dementia (Pollock, Mulsant et al. 1995). In this study the authors determined the activity of CYP2D6 (the enzyme responsible for the metabolism of most antipsychotics), identifying a 5-fold increase in adverse events (predominantly sedation and extrapyramidal symptoms (EPS) in poor metabolisers. This is of particular importance as sedation is a common side effect in people with dementia prescribed antipsychotic drugs and may be an important mediating factor of more serious secondary consequences such as oedema, pneumonia, stroke and increased risk of mortality in people with dementia (Ballard, Howard 2006).

1.4.6.1 Histamine, its relationship to antipsychotic mortality and the Histamine-N-methyltransferase polymorphism

Histamine in the CNS plays a vital role in sleep and wake, cardiovascular regulation and the maintenance of fluid balance (Haas, Sergeeva et al. 2008). Blockade of the histamine H1 receptor is a well established cause of sedation and an action of many antipsychotic drugs. Sedation in dementia is associated with prolonged antipsychotic prescription and would carry a risk of dehydration and peripheral oedema which in turn may impact mortality (Corbett, Ballard 2012). It is therefore not surprising that the H1 blocking effect of many antipsychotics has been implicated in the adverse effects associated with their use.

Histamine H1 receptor agonists disrupt sleep/wake cycles and the first antihistamines such as chlorpheniramine are strong sedatives (Nicholson, Pascoe et al. 1991). Specifically, PET imaging has demonstrated that cortical H1 antagonism is likely to underpin the sedative effect of H1

blockers (Tashiro, Mochizuki et al. 2002). H1 mRNA levels are reduced in olanzapine treated rats (Han, Deng et al. 2008) but not in those treated with the low affinity H1 antagonist antipsychotics aripiprazole and haloperidol, suggesting that histamine signalling may be affected in chronic antipsychotic treatment.

The importance of histamine blockade in people with AD is however not limited to sedation. Numerous studies in animals have demonstrated a clear link between the histamine H1 and H2 receptors and the physiological states (e.g. fluid balance) highlighted above. Histamine and the H1 and H2 agonists 2-thiazolylethylamine and 4-methylHA stimulate vasopressin and oxytocin release, which would act to increase water retention (Knigge 1999). Furthermore, 24h of dehydration increases histamine synthesis in the hypothalamus by 40% and antagonism of histamine H1 receptors decreases dehydration induced vasopressin release by 50-70% (Kjaer, Knigge et al. 1994). With respect to AD, an early study showed impaired vasopressin secretion and self-reported ratings of thirst in response to dehydration compared to age-matched controls (Albert, Nakra et al. 1989). This potentially important effect has not been studied since, nor has any interaction with histamine. This notwithstanding, alongside the research in animals linking histamine to fluid balance, it is reasonable to hypothesise that chronic blockade of brain H1 receptors would suppress vasopressin release in response to dehydration, which may be significant in a condition like AD where vasopressin is already compromised.

The first and most obvious candidate gene for modification of antipsychotic effect is clearly the H1 receptor, *HRH1*. However, there are no non-synonymous *HRH1* SNPs common enough in European samples to warrant investigation in the present cohorts (Garcia-Martin, Ayuso et al. 2009). There are a number of non-synonymous SNPs, however in a hypothesis driven approach there is not sufficient evidence, either from previous association studies or from molecular genetic approaches, to include them in the analysis.

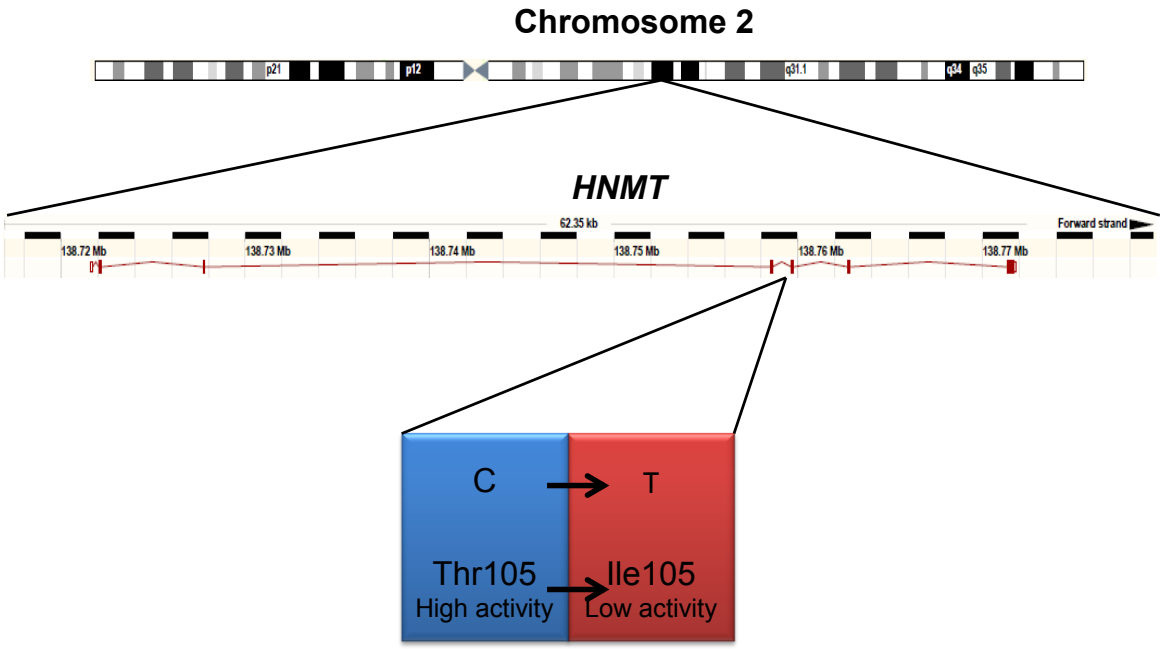

Figure 1-4 HNMT thr105ile polymorphism
Located in exon 4 of the HNMT gene located on chromosome 2. The T allele changes the amino acid at position 105 from theonine to isoleucine resulting in a less thermostable protein with a lower affinity for histamine

A second plausible candidate for genetic modification of antipsychotic associated mortality is the histamine-N-methyltransferase (*HNMT*) gene. *HNMT* is one of two enzymes (the other being diamine oxidase) responsible for termination of the actions of histamine and represents the only route to doing so in the human CNS, it is encoded by a single gene on chromosome 2 (Figure 1-4). A common c/t SNP in exon 4 of the gene has been identified which results in a threonine/isoleucine substitution at position 105 (thr105ile). This polymorphism is associated with an approximately 2-fold decrease in enzyme activity (Preuss, Wood et al. 1998, Horton, Sawada et al. 2001).

The hypothesis here is similar to that described for *COMT*, namely, a decrease in enzyme activity resulting from the rarer T allele would be associated with an increase in histamine levels and thus affect histaminergic neurotransmission, impacting key physiological process such as fluid balance and sleep. Consistent with this, *HNMT* is expressed in the prefrontal cortex and hypothalamus, two areas in which histaminergic neurotransmission is associated with wake and fluid balance respectively (Panula, Nuutinen 2013).

HNMT has been shown to significantly affect histamine neurotransmission in animal studies. In rats, inhibition of HNMT by SKF 91488, dyaminopyrimidine or metoprine has been shown to cause increases in endogenous histamine levels, a dose dependent increase in mean arterial

blood pressure and a decrease in heart rate (Duch, Bowers et al. 1978, Jochem 2002, Klein, Gertner 1981, Jochem 2004). Moreover, other animal studies have shown that SKF 91488 also induces wakefulness to a greater extent than direct injections of histamine, consistent with the hypothesis that histamine neurotransmission, mediated by HNMT is implicated in sedation (Lin, Sakai et al. 1988).

1.4.6.1.1 Section summary

Although the presence of the low activity T allele could lead to higher levels of histamine resulting in raised blood pressure due to greater activation of H3 autoreceptors, an arguably more likely hypothesis is that the more common C allele would carry the risk. In this case, higher enzyme activity would lead to reduced levels of histamine relative to T carriers, which would in turn lead to less competition with antipsychotics for H1 receptors causing more sedation. Less endogenous histamine alongside chronic blockade of H1 receptors may also affect the ability to respond to dehydration by inhibiting vasopressin release. It will be important to identify possible harmful mechanisms such as this in the prescription of antipsychotics to help inform future prescribing practice.

1.4.6.2 The extended *MAPT* haplotype and memantine treatment response

There is some suggestive evidence that memantine may inhibit and reverse the abnormal tau phosphorylation, which may contribute to its mechanism of action (see section 1.2.3.2.2).

It is not yet known whether the composition of NFTs in AD with and without psychosis differs. This gap in the literature notwithstanding, that hyperphosphorylated tau may be an important correlate of psychosis is potentially very interesting, particularly given the lack of effective treatments at present. Although the results surrounding memantine as a treatment for psychotic symptoms have been disappointing, in light of memantine's safety and tolerability profile in AD it is argued here that steps should be taken to identify the reasons why this may be. There has however been no such research to date. With evidence implicating tau in psychotic symptoms in AD and inhibition/reversal of phosphorylated tau by memantine it is proposed here that exploring

the extended tau haplotype may help explain the inconsistent findings that have arisen from the memantine literature.

There has been no research to date examining the pharmacogenetics of memantine treatment response. Tau is implicated in psychosis in AD and part of the mechanism of action of memantine may be inhibition and reversal of tau phosphorylation, thus although there are still many unknowns in this relationship, the tau haplotype may, alongside clinical trial data, help identify a subset of non-responders.

1.4.7 Methodological Issues in Genetic Association Studies of Neuropsychiatric Symptoms in Dementia

1.4.7.1 Cognitive impairment has confounded previous research on the 5HTTLPR polymorphism and psychosis

As discussed in section 1.2 there are several clinical correlates of psychosis in dementia. The most important of these is cognitive impairment, not least because it is by definition present in all participants in dementia studies.

Psychotic symptoms are more prevalent in the moderate-severe stages of dementia than the mild stages. It follows that focussing on patients with mild dementia who have not yet gone through the main risk period for the development of psychotic symptoms may be an important bias in previous research. While this has been noted as important going forward in previous reports (Proitsi, Powell 2012, DeMichele-Sweet, Sweet 2010), its impact on past research has yet to be quantified, but is essential to inform a correct interpretation.

The literature surrounding the *COMT* val158met polymorphism is simply not extensive enough to conduct an analysis of this point. Therefore a systematic review and semi-quantitative analysis was carried out on the 5HTTLPR literature to evaluate the impact of cognitive impairment on whether a significant association was identified.

1.4.7.1.1 Study Identification and Selection

Where relevant to the present review the criteria set out in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist were followed (Moher, Liberati et al. 2009).

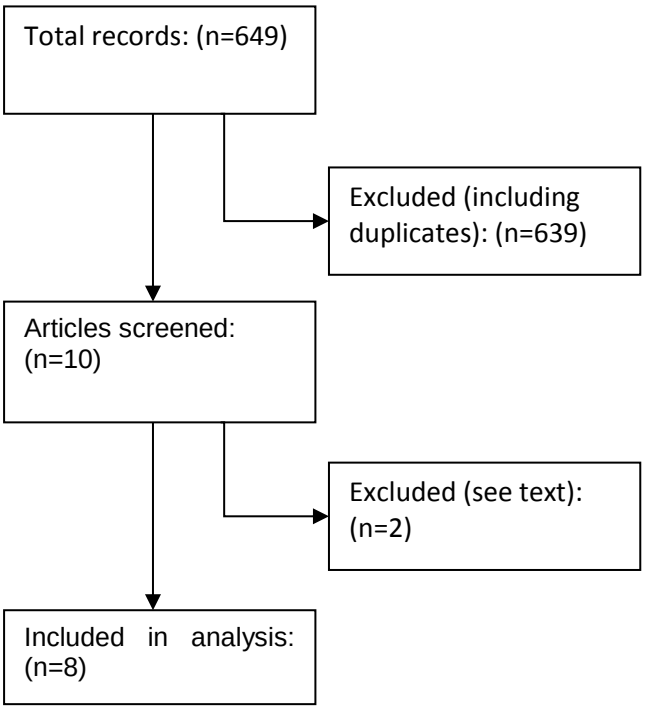

Figure 1-5 Flow diagram illustrating results of electronic and manual searches and study identification process
From (Creese, Ballard et al. 2013)

The results of the following identification process and study selection are illustrated in Figure 1-5. A search of PubMed/MEDLINE and Scopus was conducted using the search term "(dementia or alzheimer*) AND (serotonin or 5ht*) AND (polymorphism or gene*)". The only limit on the search was that articles had to be written in English. The resulting 649 records over the two lists were screened for candidate gene association studies of the 5HTTLPR polymorphism in relation to psychotic, delusional or hallucination symptoms in Alzheimer's disease. References contained in review articles and meta-analyses (DeMichele-Sweet, Sweet 2010, Borroni, Costanzi et al. 2010, Ramanathan, Glatt 2009, Flirski, Sobow et al. 2011) were also manually searched for any additional studies not picked up by the above search. Any duplicate articles were excluded.

Ten articles were found using the aforementioned method and these were screened in detail to meet the following inclusion criteria: 1) standardised diagnosis of Alzheimer's disease; 2)

psychotic symptoms (delusions, hallucinations or psychosis) assessed using standardised assessment tool; 3) level of cognitive impairment measured using MMSE and reported.

One study (Rocchi, Micheli et al. 2003) did not report MMSE, leaving nine in total. However, two were carried out by Borroni et al. in 2006 (Borroni, Grassi et al. 2006, Borroni, Grassi et al. 2006), using very similar cohorts, both reporting significant findings. In order to exclude any bias resulting from including non-independent cohorts, the Borroni et al. study with the analysis most similar to the other studies was included, that is the one that examined symptom frequencies using logistic regression rather than factor analysis (Borroni, Grassi et al. 2006), leaving eight studies in the final review (Table 1-1).

1.4.7.1.2 Results

In total, there have been eight published 5HTTLPR studies meeting the stipulated inclusion criteria (Table 1-1). Gender proportion was similar across the eight studies. Similarly, participants in all studies came from similar sources; that is, hospital outpatient clinics. Five studies employed a cross sectional design while three were carried out in prospectively studied cohorts. Other potential confounds such as age of onset and disease duration were not consistently reported across the studies so it is not possible to discuss these factors in the present review.

Table 1-1 5HTTLPR genetic association studies of psychosis

From (Creese, Ballard et al. 2013)

Study	n	Clinical setting	Females %	S allele frequency, %	Symptom	Symptom definition	Symptom frequency %	Design	MMSE[1] Score ± SD	Assessment tool	Findings
Albani [35]	235	hospital outpatients	69	48	P, D, H	P: D and H; others: score ≥1	P: NR; D: 17; H: 11	cross-sectional	18.7 ± 5.8	SBI-BP	NS
Borroni [27]	234	university centre for AD	72	53	P	D, H or misidentification present for ≥1 month	35	cross-sectional	17.6[1] (16.4 ± 6.5; 18.8 ± 6.5)	DSM-IV	SS genotype/ S allele
Ha [36]	65	hospital clinic	59	75	D	present/ absent	66	cross-sectional	15.2 ± 5.6	BEHAVE-AD	NS
Pritchard [34]	367	memory clinic	56	57	P, D, H	P: D and H at any stage; others: present at any stage	P: 39; D: 62; H: 44	prospective	18.6 ± 4.2	NPI	NS
Proitsi [29]	1,008	secondary care services	72	42	P	P: CFA[4] model, D and/or H	N/A: quantitative NPI score	cross-sectional	12.8 ± 8.8	NPI	SS genotype *COMT G interaction
Quaranta [30]	148	university neuropsychology unit	68	47	P	D or H present for ≥1 month and NPI ≥2	35	cross - sectional	15.9[1] (13.8 ± 4.6; 18 ± 5.7)	NPI + clinician	LL genotype/ L allele
Sweet [31]	332	outpatients and community	64	52	P	D or H	43	prospective	13.8 ± 7.2	E-BEHAVE-AD + CBRS	LL genotype/ L allele
Ueki [37]	200	NR	67	89	D, H	present at any stage	D: 22; H: 12	prospective	19.2[1] (19 ± 3.5; 19.4 ± 3.3)	BEHAVE-AD	NS

[1] Overall mean MMSE not reported; figure derived from MMSE of psychotic and non-psychotic participants, respectively (in parentheses). P = Psychosis; D = delusions; H = hallucinations; CFA = confirmatory factor analysis; NS = not significant; NR = not reported.

As shown in Table 1-1, four out of eight studies reported a significant association between the 5HTTLPR polymorphism and psychotic symptoms in dementia (Borroni, Grassi et al. 2006, Quaranta, Bizzarro et al. 2009, Proitsi, Lupton et al. 2010, Sweet, Pollock et al. 2001); two found the LL genotype/L allele to be associated while one found the SS genotype/S allele associated and the last reported only an interaction between SS and the absence of *COMT* val to be associated. Six of the eight investigated a psychotic phenotype of some kind; definitions are listed by study in Table 1-1. Of the remaining two studies not using a psychosis phenotype, one investigated delusions only while another investigated delusions and hallucinations separately, in each case no significant association was found (Albani, Prato et al. 2009, Ueki, Ueno et al. 2007).

Allele frequencies were distributed as expected according to ethnicity (Gelernter, Kranzler et al. 1997, Kunugi, Hattori et al. 1997). Among the six European ancestry cohorts (Sweet 2001, Borroni, Grassi et al. 2006, Albani, Prato et al. 2009, Quaranta, Bizzarro et al. 2009, Pritchard, Pritchard et al. 2007, Proitsi, Lupton et al. 2010) the S allele was carried by 42-57% of individuals

while this figure was much higher at 75 and 89% in the two Asian cohorts (Ha, Cho et al. 2005, Ueki, Ueno et al. 2007). Neither of the Asian studies reported a significant association, leaving the four previously mentioned significant studies among the six European cohorts.

The median MMSE score of the eight studies included was 16.75. On examination of mean sample MMSEs plotted according to whether or not the result was significant (Figure 1-6 below), it can be seen that those studies that have reported a significant finding have on the whole lower MMSE scores than those which report no association. Specifically, three out of four studies with a mean MMSE of below 16.75 reported a significant finding, while one out of four above 16.75 reported a significant association.

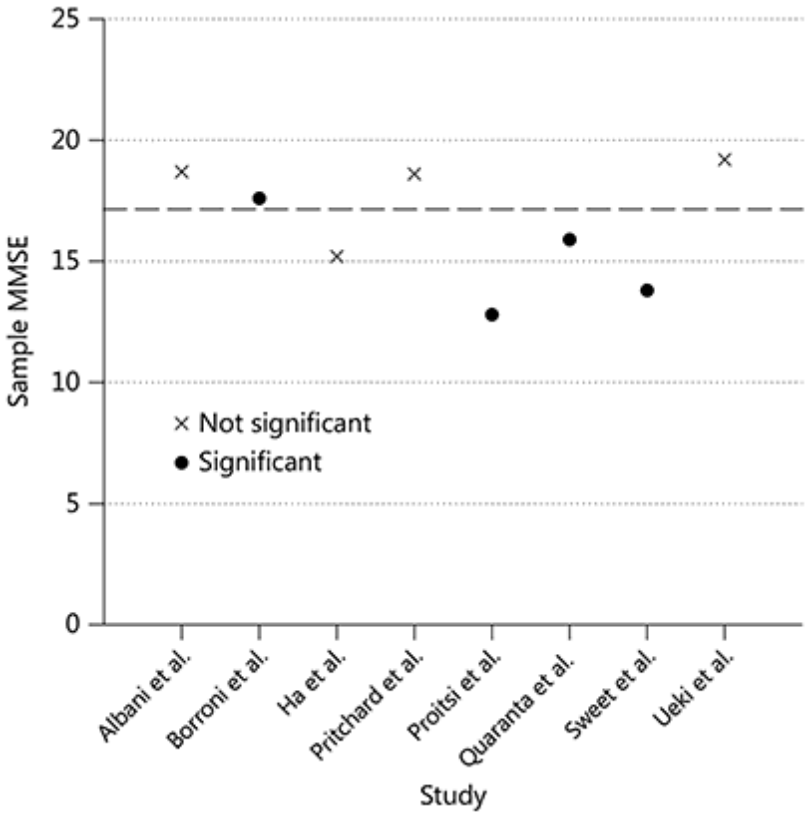

Figure 1-6 5HTTLPR psychosis studies plotted by MMSE and whether or not a significant finding was reported

Dotted line represents median MMSE of the 8 studies included (16.75). From (Creese, Ballard et al. 2013)

Four of the eight studies incorporated disease severity into their statistical analyses (Quaranta, Bizzarro et al. 2009, Borroni, Grassi et al. 2006, Proitsi, Lupton et al. 2012, Pritchard, Pritchard et al. 2007), and three of these reported a significant finding, two for the SS genotype (Proitsi,

Lupton et al. 2010, Borroni, Grassi et al. 2006) and one for the LL genotype (Quaranta, Bizzarro et al. 2009). The one study not reporting an association (Pritchard, Pritchard et al. 2007) had a sample with a relatively high MMSE (18.6 ±4.2). Of the remaining four which did not incorporate MMSE into their analysis (Albani, Prato et al. 2009, Ha, Cho et al. 2005, Sweet, Pollock et al. 2001, Ueki, Ueno et al. 2007), one reported a significant finding (Sweet, Pollock et al. 2001) and this study was one of two which had a sample MMSE of lower than 16.75 (the other being (Ha, Cho et al. 2005)).

If the two Asian studies are excluded, all three of the remaining lower MMSE studies and one of the three remaining higher MMSE studies report significant associations (the median remains the same at 16.75).

1.4.7.1.3 Discussion

This review quantifies the extent to which cognitive impairment (measured by MMSE) may have acted as a confounding variable in the literature surrounding genetic association studies of the 5HTTLPR polymorphism in AD psychosis. Three out of four studies with a sample MMSE below the median of 16.75 reported a significant finding compared with one out of four among those above. This brings greater clarity to the current understanding of the role of the 5HTTLPR polymorphism and suggests that it is a risk factor for psychosis in people with AD. This conclusion is strengthened by the fact that three of the four studies which factored MMSE into their analyses reported a significant association.

The exploration of other potential clinical and demographic confounding variables was limited by the inconsistent reporting among previous studies. However, it is useful to discuss these as they are relevant to the studies examining the role of *COMT* val158met as well.

Those variables that were able to be reviewed were gender, the source of participants and ethnicity. The former two appeared to be broadly similar among all the 5HTTLPR studies reviewed. Conversely, both of the Asian studies failed to report a significant association and this may have been driven by the substantially higher frequency of the S allele among these populations. When excluded from the review, although two studies were lost in doing so, the

relationship between cognitive impairment and significant association did strengthen: all three lower MMSE studies were positive, while only one out of three higher MMSE studies was positive. Ethnicity has been highlighted as important in previous, more general reviews, but as the first review specifically of this polymorphism it is possible to refine the initial conclusion and propose that a relationship between 5HTTLPR and psychosis may not exist in the Asian population and that among Europeans the relationship may only be present among those with greater cognitive impairment.

What has been highlighted here is the importance of consistency in phenotypic definition and perhaps that psychotic symptom phenotypes in AD should be described with reference to cognitive impairment. With these considerations, more work is needed to fully determine the role of 5HTTLPR in psychosis in AD

There is evidence that individual psychotic symptoms differ in their underlying neurobiological associations (Forstl, Burns et al. 1994) as well as their clinical presentation (Ballard, Bannister et al. 1995) and it is therefore vital that in future suitable phenotypes are identified and used consistently. The evidence presented here suggests cognitive impairment should be considered as part of this phenotype but there is less scope to draw firm conclusions about the definitions of the psychotic symptoms themselves. Although all studies were selected on the basis of their investigation of delusions, hallucinations or psychosis, the phenotype definitions did vary somewhat. The two Asian cohorts were the only two that did not investigate a psychosis phenotype it is therefore not possible to say whether it is specifically a combined phenotype that is related to 5HTTLPR, and if so susceptible to moderation by cognitive impairment, rather than individual symptoms as ethnicity that may have been driving the results here. A further constraint to this interpretation is that only two of the six European studies reporting on a psychosis phenotype also reported on delusions and hallucinations separately. Although in each case this did not change the results (they both remained negative), the fact that it is two studies alone makes it impossible to draw conclusions about the relationship between 5HTTLPR, individual psychotic symptoms and cognitive impairment.

Prospectively assessed cohorts offer the best opportunities for accurate symptom classification. They offer far more accurate differentiation between transient psychotic-like phenomena such as

delirium and persistent psychosis; however their use in AD psychosis genetic research, presumably largely due to practical constraints, is limited. There is evidence that multiple or recurrent psychotic symptoms are more heritable than single symptoms occurring at any assessment (61% and 30% respectively) (Bacanu, Devlin et al. 2005). However, even among those studies reviewed here which employed prospective designs the multiple and/or recurrent definition was not adopted, potentially leaving non genetic factors to play a much greater role in the phenotype, a clear issue when aiming to establish the role of genetic variability in a symptom.

What cannot be disentangled based on this review is why half of the significant studies reported the L allele as associated with psychosis, one reported the association to be with the S allele and one reported only a significant interaction with the *COMT* val158met polymorphism. There are two possible sources of this variation. The first is differences in the sample characteristics, with the most obvious source being phenotype definition. Unlike Sweet et al. (2001) and Quaranta et al. (2009) (who both report the LL genotype as the risk), Proitsi et al. do not stipulate any exclusion criteria which would mitigate the impact of transient episodes of delirium on NPI symptom score in their study. Delirium is common in dementia and unless participants are assessed serially or measures are taken to account for delirium there is a risk that participants could wrongly score highly on ratings of psychotic symptoms, particularly hallucinations. This is further complicated by research showing that delirium accelerates cognitive decline in dementia (Fong, Jones et al. 2009). Therefore there may be different biological mechanisms underlying delirium and psychosis which may give rise to the different results here. The second source of variation is at the genetic level and this will be discussed in greater detail in section 7.2.2 of the General Discussion.

It should be acknowledged that this review has only been conducted on eight studies and due to the substantial differences between them with regard to methodology and analysis it was not possible to conduct a meta-analysis, therefore the conclusions should be tempered by this. Although it was not possible to statistically test this relationship, what is described extends the findings from a substantial number of studies highlighting more severe cognitive impairment as a risk for psychosis in AD and demonstrates that this is likely to have influenced the results of previous genetic association studies of 5HTTLPR.

The aim of this analysis was to quantify the issue of cognitive impairment in genetic studies of the 5HTTLPR polymorphism and psychotic symptoms in AD, which has been the subject of much discussion previously. In summary, this review highlights that most of the significant findings of studies of 5HTTLPR and psychosis in AD have been carried out in cohorts with more severe cognitive impairment. All of the arguments presented above relating to phenotypic definition are applicable to the studies examining the *COMT* val158met as well.

In order to accurately describe the relationship between 5HTTLPR, and other polymorphisms, and psychosis, future studies should consider the cognitive impairment of their sample, select non-psychotic controls accordingly and interpret their findings with reference to the level of cognitive impairment of the sample.

1.4.7.2 *COMT* val158met, 5HTTLPR and cognitive decline in Alzheimer's disease

As stated in section 1.4.2 the role of *COMT* val158met and 5HTTLPR in psychotic symptoms in dementia may not be direct. Dopamine and serotonin are involved in a wide variety of physiological functions including reward, depression, sleep, eating and, importantly, cognition. Cognitive decline is a well-established correlate of psychosis in AD and it will be important to elucidate whether and to what extent *COMT* val158met and 5HTTLPR are involved in cognitive decline. Not only will this bring a more complete understanding of the genetic correlates of psychotic symptoms it may also explain why faster cognitive decline is a correlate, that is to say genetic polymorphisms in AD patients with psychotic symptoms may be responsible.

Both the *COMT* val158met and 5HTTLPR polymorphisms have been implicated in cognitive processes and performance. In the case of *COMT* val158met, there is a much larger body of literature implicating it in cognitive performance than psychosis. It is surprising then that it has been the focus of so little research in dementia, particularly in light of the relationship between cognitive impairment and psychotic symptoms. However, there has been some focus on *COMT* val158met in PD.

In a relatively large study of 288 PD patients, Foltynie et al. (2004) reported a dose dependent decrease in executive function, specifically planning ability, with each met allele carried (Foltynie,

Goldberg et al. 2004). Interestingly the direction of this relationship is opposite to what has been found in healthy adults and those with adult psychotic disorders, where the met allele has been associated with better cognitive performance than the val allele (Basterra, Sanchez-Torresa et al. 2011, Malhotra, Kestler et al. 2002), although not consistently (Wardle, de Wit et al. 2013). However, in a follow up study by the same group, there was no effect of genotype on MMSE decline over 5.2 years (averaging 0.3 points per year), but they did replicate their previous finding of impaired executive function being associated with the met allele in an independent cohort of individuals (N=138) (Williams-Gray, Hampshire et al. 2008).

The *COMT* val/val genotype has been associated with decline in both executive functioning and declarative memory in cognitively normal adults over a five year period (de Frias, Annerbrink et al. 2004, de Frias, Annerbrink et al. 2005). However, two other studies examining decline over two and ten year periods failed to find a effect on decline although one did report an average overall worsening of performance average across the five years among val/val genotypes, particularly on a measure of working memory (Starr, Fox et al. 2007, Erickson 2008).

Findings from individuals with 22q11.2 deletion syndrome add further strength to the hypothesis that dopamine levels mediated by *COMT* genotype impact cognitive performance. Individuals with 22q11.2 deletion syndrome only carry one copy of the *COMT* due to a micro-deletion on chromosome 22 which includes the entire gene. Gothelf et al. (2005) measured cognition during late childhood and early adolescence and evaluated the impact of the *COMT* val158met polymorphism in a group of 22q11.2 deletion syndrome individuals alongside others with other developmental disabilities (Gothelf, Eliez et al. 2005). They found that the *COMT* met genotype predicted a greater decline in cognitive ability in 22q11.2 deletion syndrome participants, but not in the control group. These individuals would probably have had a lifelong increase in dopaminergic tone, resulting from reduced expression of *COMT*, which appears to have been exacerbated the low activity met allele. Taken with the results from healthy adults, the suggestion here is that too much or not enough dopamine signalling in the PFC can negatively impact cognition, consistent with an inverted U shaped curve and may have implications in neurodegenerative diseases where dopaminergic neurotransmission is affected.

There has only been one study of the relationship between 5HTTLPR and cognitive decline. In this study of 411 older adults, without dementia, no effect was reported. There was however an association between a variable number tandem repeat (VNTR) polymorphism in *SLC6A4* which is also known to affect transcription. The 12 repeat homozygous genotype (also associated with higher transcription) was associated with a decrease in fluid intelligence, memory and aggregated general cognition score over a 15 year period (Payton, Gibbons et al. 2005).

Unlike dopamine, serotonergic deficits have received some attention in the AD literature surrounding post-mortem correlates of cognitive decline. Density of $5HT_{2A}$ receptors in the temporal cortex (BA21) is negatively correlated with rate of cognitive decline in AD (Lai, Tsang et al. 2005). An earlier study also found density of $5HT_{1A}$ receptors in the frontal cortex (BA11) to be positively correlated with cognitive decline (Lai, Tsang et al. 2002). Lai et al. (2002) also demonstrated that serotonin levels in the frontal cortex were negatively correlated with cognitive decline. The 5HTTLPR L allele may fit into this picture by exacerbating the already depleted levels of serotonin associated with cognitive decline, due to the increased in 5HT reuptake sites associated with this allele. One must be careful not to imply causation, the Lai et al. (2005) study only reported a correlation, but if an association with 5HTTLPR was found it would bring greater clarity to this hypothesis as it would provide a functional serotonergic association with cognitive decline.

1.4.7.2.1 Section summary

In summary, there have been no post-mortem investigations into the role of dopaminergic neurotransmission and cognitive decline in AD. However, there is evidence from a wider body of genetic association studies that the *COMT* val158met polymorphism is associated with cognitive decline in healthy adults, those with neuropsychiatric syndromes and more broadly cognitive performance in PD. Conversely, the literature surrounding 5HTTLPR and cognitive decline is sparse; the only study conducted to date failed to find an association but there evidence from post-mortem studies in AD implicating 5HT in cognitive decline. Frontal cortical serotonin concentration and $5HT_{1A}$ receptor density and $5HT_{2A}$ receptor density in the temporal cortex are associated with rate of cognitive decline in AD. Given that cognitive impairment may have confounded previous research into 5HTTLPR and psychosis it would be prudent to consider the

role of both polymorphisms in cognitive decline in AD. Indeed this would be essential in order to fully explore the relationship of these two polymorphisms and psychosis in AD.

1.5 Summary

- Delusions and hallucinations are common in AD and DLB/PDD but their aetiology is not well understood.
- Candidate gene association studies are a useful tool in advancing understanding in this area. Three widely studied polymorphisms, 5HTTLPR, *COMT* val158met and the extended *MAPT* haplotype, will form the basis of the work presented here.
- Both 5HTTLPR and *COMT* val158met are implicated in cognition, which is a close correlate of psychosis in dementia, although this relationship has yet to be investigated in AD.
- Treatment options of psychotic symptoms are currently very limited. Two that are in use in AD, antipsychotics and memantine, have mixed efficacy and in the case of antipsychotics there are serious safety concerns.
- The mechanisms underlying the safety concerns associated with antipsychotic use in AD are not well understood but it is hypothesised that sedation via antagonism of histamine H1 receptors is an important property.

1.6 Aims

- To examine the relationship between the 5HTTLPR and *COMT* val158met polymorphisms and delusions and hallucinations in AD and DLB/PDD in cohorts. Patient data will be well characterised for these symptoms in order to provide a more complete understanding of the common mechanisms that may underlie psychosis across the AD, DLB/PDD spectrum.

- To establish whether either of the above polymorphisms are associated with rate of cognitive decline in AD in order to bring greater clarity to current understanding of their role in the aetiology of psychotic symptoms.

- To examine the course of delusions and hallucinations in AD and their relationship to the extended *MAPT* haplotype in order to evaluate whether tau is associated with a more severe course of AD, specifically with respect to psychotic symptoms.

- Establish the extent to which the histamine acting antipsychotics and the *HNMT* polymorphism affect mortality in AD.

- Conduct a preliminary exploration of the mechanisms that underpin efficacy in memantine as a treatment for psychosis through the study of the extended *MAPT* haplotype.

Chapter 2 General Materials and Methods

2.1 Description of cohorts

Clinical data and samples from dementia patients were obtained from eleven different sources, these are summarised in Table 2-1 and described in detail from 2.1.1 onwards.

Table 2-1 Overview of Sample Sources

Source	Source Type	DNA Sample Type	N[1]
MAIN-AD	RCT	Buccal swab	77
MAGD	RCT	Buccal swab	34
OPTIMA	Prospective cohort study	Brain	148
Norway	Prospective cohort study	Buccal swab	63
WHELD	RCT	Buccal swab	70
DEMVEST	Prospective cohort study	Blood	151
Greek	Outpatients	Buccal swab	121
ARUK	Prospective cohort study	Buccal swab/Brain	305
DCR	Prospective cohort study	Buccal swab	158
ANM	Prospective cohort study	Buccal swab/blood	260
Brain banks	Prospectively followed individuals	Brain	21
TOTAL			**1408**

[1]Reflects the total number of patients available for screening for use in studies presented in the results chapters

Abbreviations: MAIN-AD (Memantine for Long-Term Treatment of Neuropsychiatric Symptoms in Dementia); MAGD (Memantine for Agitation in Dementia); OPTIMA (Oxford Project to Investigate Memory and Aging); Norway (The Course of Dementia, Neuropsychiatric Symptoms and Drug Use and its Impact on Health Service Resource Use); WHELD (Improved Well-Being and Health for People with Dementia); DEMVEST (Dementia Studies in West Norway); ARUK (Alzheimer's Research UK); DCR (Dementia Case Register); ANM (Addneuromed)

In addition there were a total of 113 cognitively and neurologically normal controls, screened for absence of dementia drawn from the ANM and DCR cohorts and a study of post operative cognitive decline (the POCD cohort) (Ballard 2012).

2.1.1 Memantine for Long-Term Treatment of Neuropsychiatric Symptoms in Dementia (MAIN-AD)

A 24 week randomised, placebo controlled clinical trial evaluating whether memantine is safer and more effective than neuroleptics for the treatment of neuropsychiatric symptoms in Alzheimer's disease.

Written informed consent was obtained for two hundred participants recruited from four sites in the UK (London, Essex, Oxford and Newcastle) and one in Norway.

Inclusion and exclusion criteria for the study are listed below (from the MAIN-AD trial protocol):

Inclusion criteria:

- Living in a nursing or social care facility.
- Fulfill the NINCDS/ADRDA criteria for possible or probable Alzheimer's disease (AD).
- Taking at least 0.5mg daily of haloperidol, 0.5mg daily of risperidone, 5mg daily of olanzapine or 25mg daily of quetiapine or another neuroleptic, which in the opinion of the responsible clinician could be safely converted to one of these neuroleptics, for a minimum of 3 months prior to entry into the study.
- If taking a cholinesterase inhibitor, it has been prescribed for at least 6 months before the date of assessment, with a stable dose for at least 3 months.
- Not taking anticonvulsants other than carbamazepine or sodium valproate. The use of either of these 2 agents is permissible if the dose has been stable for at least 4 weeks.
- If taking any other psychotropic drugs (e.g. antidepressants, benzodiazepines, chlormethiazole), that the dose has been stable for at least 4 weeks prior to randomization.
- Not currently or in the past 6 weeks receiving treatment with memantine and a responsible clinician not considering treatment with memantine.
- Not taking any medications that are contra-indicated or not recommended in combination with memantine, as defined in the British National Formulary, including ketamine, dextromethorphan, amantidine.

- Written informed consent provided by the participant's next of kin or a legal representative.

Exclusion criteria:

- Clinician responsible for care, or study clinician considers that the patient suffers from any physical condition (including marked extra-pyramidal disorder), which would make participation in the trial distressing or likely to increase suffering.
- Patients with a systolic blood pressure whilst sitting greater than 180 mm/Hg or less than 90 mm/Hg, or a diastolic blood pressure whilst sitting greater than 100 mm/Hg or less than 50 mm/Hg at the screening visits or baseline.
- Patients with a recent history (within 3 months of screening) or currently untreated B12 or folate deficiency.
- Patients with a recent history (within 3 months) or untreated clinically significant hypothyroidism or hyperthyroidism (patients with thyroid disease may be included provided they are euthyroid and stable).
- Severe aggression (≥8) on item 3 of the NPI subscale, with aggression as the predominant symptom.
- Patients with psychotic DSM IV TR Axis 1 disorder other than in the context of Alzheimer's disease, including schizophrenia, schizoaffective disorder and bipolar disorder.
- Participants known to have sensitivity to memantine, amantadine, rimantidine or lactose.
- A current diagnosis of primary neurodegenerative disorders other than Alzheimer's disease for example Huntington's disease, Parkinson's disease, etc.
- Uncontrolled epilepsy.
- Current evidence of delirium.
- Severe renal impairment, as measured by or equivalent to an estimated creatinine clearance of < 5 mL/min/1.73m^2
- Severe hepatic impairment.
- Unable to swallow tablets or capsules.
- Low probability of treatment compliance.
- Currently taking memantine.

- Memantine during the 6 weeks prior to randomization.
- Previous evidence of lack of efficacy or tolerability to memantine.
- Taking any of the following substances:
    - An investigational drug during the 4 weeks prior to randomization.
    - A drug known to cause major organ system toxicity during the 4 weeks prior to randomization.
    - Started any new psychotropic medication during the 4 weeks prior to randomization. Participants who have been on a stable dose of psychotropic during the 4 weeks prior to randomization are still eligible.
    - Other N-methyl-D-aspartate (NMDA) antagonists: amantadine, ketamine, and dextromethorphan.
    - Barbiturates and primidone.
    - Baclofen and dantrolen.
    - Antimuscarinics.
    - Anticonvulsants other than sodium valproate or carbamazepine. These 2 agents were permissible if doses had been stable for at least 4 weeks.

Following medical screening, individuals eligible for the study were randomised to either continue their current dose of neuroleptic medication (or similar) or stop their current dose of neuroleptic and commence memantine (10 mg twice daily, or 10 mg once daily for individuals with impaired renal function).

Assessments were carried out at four time points during the 24 week period; 0 (baseline), 6 weeks, 12 weeks and 24 weeks (end of study). Medication history was also recorded at baseline from GP records and during the study from nursing home medication administration records. Similarly, relevant medical history was recorded from GP notes and records were kept during the study of any newly developed co-morbidity. A proportion of London participant visits and general trial administration (submitting amendments, information for the reporting of serious adverse events) was carried out by Byron Creese.

In November 2010, approximately one year after the start of recruitment to the clinical trial itself, I submitted a substantial amendment to the MAIN-AD Research Ethics Committee (REC) to allow

collection of buccal mucosa cells from participants enrolled at the UK study sites. This study is therefore covered under the original MAIN-AD REC approval. This sub-study was adopted into the UKCRN portfolio and support was obtained from the Thames Valley and North East branches of the Dementias & Neurodegenerative Diseases Research Network (DeNDRoN). In total, samples were obtained from 77 individuals from the London, Oxford, Essex and Newcastle sites. Training in the collection of samples was provided to all additional research staff. DeNDRon staff at the Oxford and Newcastle site were responsible for consenting and collecting samples. At the Essex site, North Essex Partnership Foundation Trust carried out consenting and some sample collection. Some sample collection at the Essex site, and all consenting and sample collection at the London site was carried out by me.

2.1.2 Memantine for Agitation in Dementia (MAGD)

Collaboration with Dr Chris Fox, University of East Anglia

This cohort is described in detail in Fox et al. (2012). In summary, this was a 12 week randomised, placebo controlled clinical trial into whether memantine is a safe and effective treatment for agitation in dementia. Participants were recruited according to the below inclusion/exclusion criteria (Fox, Breitner et al. 2012):

Inclusion criteria:

- Residential/Inpatients at recruitment to the study with a history of at least 2 weeks behavioural disturbance.
- Alzheimer's disease only as per NINCDS-ADRDA criteria and Haschinski Score<=4.
- Moderately severe to severe Alzheimer's disease (baseline MMSE>=4)
- Clinically significant agitation that requires treatment.
- Severity of agitation defined by Cohen Mansfield agitation inventory (CMAI) > =45.
- Age >= 55.

Exclusion criteria:

- Memantine usage in the 4 weeks prior to the start of the study.

- Cholinesterase inhibitor for less than 3 months and not on a stable dose.
- Antipsychotic, anti-epileptic, antidepressant, benzodiazepine, lithium or hypnotic dosage alteration in the 2 weeks prior to the start of the study.
- Anti-parkinson medication.
- Hypersensitivity to memantine or any of the excipients in the formulation.
- Severe renal impairment
- Epilepsy, history of convulsions or seizure, or receiving any anti-epileptic treatment.
- Concomitant usage of N-methyl-D-aspartate (NMDA) antagonists such as amantadine, ketamine or dextromethorphan.
- Recent myocardial infarction, uncompensated congestive heart failure and uncontrolled hypertension.
- Severe, unstable or poorly controlled medical illness.
- Any disability that may interfere with the patient completing the study procedure. Active malignancy.
- Delirium, pain or any medical illness as a clear cause of agitation.
- Any important drug interactions: Prohibited during study and in the 14 days preceding enrolment/inclusion are: Analgesic dextromethorpan mentioned above Dopaminergics-amantadine mentioned above warfarin due to theoretical International Normalised Ratio prolongation.

One hundred and fifty three participants who met the inclusion/exclusion criteria above and passed medical screening were recruited from five UK centres (Oxford, Plymouth, South East London, Kent and East Anglia) and randomised to either memantine 10 mg twice daily or placebo for 12 weeks. Assessments were carried out at baseline, 4, 6 and 12 weeks.

Dr Chris Fox (MAGD Chief Investigator) gave his support for a substantial amendment to be submitted (by me) to the MAGD REC to allow collection of buccal mucosa cells from participants in the study. The amendment was approved in November 2010, several years after recruitment first started into the trial. This study is therefore covered under the original MAGD REC approval. The sub-study was adopted into the UKCRN portfolio and support was obtained from the Kent and South East London/South East Mental Health Research Network (MHRN) hubs and South West, Thames Valley and East Anglia DeNDRoN. Training was provided to all staff. DeNDRoN

staff at these five research sites took consent and samples from participants. In total samples were obtained from 34 individuals.

2.1.3 Oxford Project to Investigate Memory and Aging (OPTIMA)

Samples from this source are covered by REC project reference 10/H0703/30. On enrolment, participants in OPTIMA consented for their tissue to be used in research. As part of OPTIMA participants underwent detailed clinical evaluations approximately every six months until death. Information on medication was also collected at most visits.

I wrote an application to OPTIMA for DNA samples relating to cases which met the below inclusion/exclusion criteria:

Inclusion criteria:

For pathologically confirmed samples:

- Meet the Consortium to Establish a Registry for Alzheimer's disease (CERAD) criteria for probable or definite Alzheimer's disease (Mirra, 1991) or the National Institute of Aging (NIA-REAGAN) criteria for high likelihood of Alzheimer's disease (Hyman et al., 1997);

Or for clinically diagnosed samples:

- Operationalised clinical diagnosis of AD at death made using National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable Alzheimer's disease (McKhann et al., 1984).

Exclusion Criteria:

- Any patients not meeting the criteria listed for each disease set out under inclusion criteria.

The criteria were kept deliberately wide due to the different experiments planned, thus allowing the exclusion of certain cases as and when dictated by each experiment.

The project was approved and DNA and accompanying clinical data was supplied for 148 patients in total.

2.1.4 The Course of Dementia, Neuropsychiatric Symptoms and Drug Use and its Impact on Health Service Resource Use (the Norway cohort)

Collaboration with Dr Geir Sælbeck and Dr Sverre Bergh, Innlandet University Hospital Trust

In this ongoing prospective cohort study participants who met the following criteria were eligible for inclusion:

Inclusion criteria:

- Nursing home patients with a planned stay of more than four weeks.
- Dementia, as defined in the International Classification of Diseases version 10 (ICD-10).

Exclusion criteria:

- Not able to obtain buccal swab samples for DNA extraction.

Buccal swab kits were provided to the research team in Norway who collected the samples and returned them to KCL for DNA extraction and storage see section 2.4.

Assessments are carried out at baseline and at 3, 6, 12, 18, 24 and 36 months. At the time of this project a maximum of six months of data was available.

2.1.5 Improved Well-Being and Health for People with Dementia (WHELD)

This study was an RCT evaluation of a variety of non-pharmacological interventions on quality of life and psychotropic drug use in dementia. Individuals were eligible for this study provided that they were residing in participating care homes and scored ≥1 on the Clinical Dementia Rating Scale (CDR) and ≥4 on the Functional Assessment Staging Tool (indicating mild dementia, see section 2.2.3.2) and met NINCDS-ADRDA criteria for probable or possible AD. This was a three centre study with sites at Oxford, South East London and North East London.

Participants were assessed on a variety of measures at baseline and 9-12 months after.

Buccal swab samples were covered under REC approval 10/H0703/30. That is, unlike MAIN-AD and MAGD, sample collection was not a sub-study of WHELD. Ethical approval was obtained in early 2012. Although it was a separate study to the WHELD RCT, ethical permission was obtained to access the WHELD participant database to identify participants. I carried out the consenting and sample collection for all participants, at all three sites and some patient assessments at the South East London site.

2.1.6 Dementia Studies in West Norway (DEMVEST)

Collaboration with Prof. Dag Åarsland, University of Oslo and Karolinska Institute

The DEMVEST cohort is an existing collection of DNA samples from patients in Norway stored at KCL and extracted from blood collected from individuals with dementia who were assessed at baseline and at 12 months. The cohort contains AD, DLB and PDD patients.

2.1.7 Greek Cohort

Collaboration with Prof. Magda Tsolaki, Aristotle University of Thessaloniki and Dr John Powell, KCL

Individuals were AD patients and attendees at hospital outpatients' clinics. They received regular assessments but because it was not part of a particular project the frequency of assessments varied somewhat as did the length of follow up for each patient. However the entire database was requested and the specific inclusion/exclusion criteria for each study in the results chapters was applied as required. Medication history was available for most patients. Dates of death were not originally available but were obtained on request from Dr Mariana Siapera in Greece.

2.1.8 Alzheimer's Research UK (ARUK) and Dementia Case Register (DCR)

Collaborations with Prof. Simon Lovestone, Dr Rob Stewart and Dr John Powell, KCL

Individuals recruited into these cohort studies were assessed at yearly intervals on a wide range of measures. In general individuals were followed up for a maximum of 4-5 years. Dates of death and medication were recorded for most individuals however they were not entered onto the electronic data base. Therefore, paper files for every case were screened and this information recorded.

2.1.9 Addneuromed (ANM)

Collaboration with Prof. Simon Lovestone and Dr John Powell, KCL

The wider aims of this project are to identify AD biomarkers and improve experimental models for AD biomarker discovery. DNA samples were collected from six sites in Europe (University of Kuopio, Finland; University of Perugia, Italy; Aristotle University of Thessaloniki, Greece; King's College London, UK; University Hospital Toulouse, France and University of Lodz, Poland) and are stored at KCL. Individuals were followed up every three months for one year at all sites, while some of those at the London site continued to be followed up at yearly intervals thereafter up to a maximum of 8 years.

Dates of death were not routinely collected as part of the study however the PIs for each site were contacted and asked for the reasons for withdrawal and dates of death for everyone who did not complete one year of assessments.

2.1.10 Institute of Psychiatry and Newcastle brain banks

Samples from this source are covered by REC approval 08/H1010/4. Requests were made to each brain bank for donors meeting the following criteria:

- Diagnosis of DLB or PDD according to McKeith et al. (2005) criteria (DLB) or UK Parkinson's Disease Society Queen Square Criteria for PD and DSM-IV criteria for dementia (PDD).
- Serial assessments for at least six months of psychotic symptoms using a standardised validated tool.
- MMSE at last assessment available.

2.2 Clinical assessments

2.2.1 Psychotic symptoms

2.2.1.1 Neuropsychiatric Inventory (NPI)

The NPI (Cummings 1997) is a questionnaire which assesses the following 12 behavioural symptoms in dementia: delusions (persecutory or misidentifications), hallucinations (visual, auditory or tactile), agitation/aggression, anxiety, depression, apathy, disinhibition, euphoria, irritability/lability, aberrant motor disturbances, sleep disturbances and appetite/eating disorders.

It is administered by a researcher in an interview style with a care giver who has an appropriate level of contact with the participant. The interviewer asks whether the participant has experienced each symptom at all over the past four weeks. If the symptom is not present the interviewer moves on to the next symptom. If the response is positive the interviewer uses probing questions to clarify the nature of the symptom and then determines if indeed it should be coded as present. For example, a carer may report that the participant has experienced hallucinations over the past 4 weeks; however on further questioning it may become apparent that they were discreet hypnagogic hallucinations, which should not be counted as it is unlikely that these are associated with any pathological processes.

If the interviewer decides it should still be coded as present the carer is then asked how often the symptom occurs on a scale of 1 (occasionally) to 4 (every day) and how severe it is, when it does occur on, from 1 (mild) to 3 (severe). These frequency and severity scores are then multiplied to give a composite NPI score for that symptom, the highest possible score being 12 (with scores of 9, 8, 6, 4, 2, 1 and 0 falling below).

The questionnaire also contains a distress score for each symptom where the carer is asked how distressing the symptom is on a scale of 0 (not at all) to 5 (extremely/severely).

This scale was used in the following cohorts: MAIN-AD, MAGD, Norway, DEMVEST, Greek, ARUK, ANM, DCR, and WHELD.

2.2.1.2 Cambridge Mental Disorders of the Elderly Examination (CAMDEX)

CAMDEX (Roth, Tym et al. 1986) is an interview schedule which, alongside measuring cognitive impairment, assesses the extent of behavioural and psychological morbidity according to interviewer observations (10 items) as well as in interviews with both the participant (37 items) and their main carer (18 items). The items on the scale rate the presence of delirium/confusion, depression, sleep disturbances, anxiety, and delusions and hallucinations. For psychotic symptoms specifically, ratings from the informant section of the scale were used, in keeping with the other assessment scales used in this project. Psychotic symptoms are covered by one item for hallucinations and one item for persecutory delusions, rated in a binary fashion. Although the questions only refer to the patient's present state, longitudinal assessment with this scale allows persistence to be rated. It also has sections which allow the interviewer to collect information on co-morbidities, past medical history and current medication.

This scale was used in the OPTIMA cohort.

2.2.1.3 Present Behavioural Examination (PBE)

The PBE is a detailed examination, administered by an investigator, of the presence of neuropsychiatric symptoms over the four weeks prior to the assessment (Hope, Fairburn 1992). Questions relating to psychotic symptoms specifically are covered by one question for hallucinations (covering auditory or visual), one question for grandiose delusions and one question for persecutory delusions. One mandatory question for each symptom is asked which is followed up with further questioning to ascertain qualitative information and the frequency of the symptom, on an ordinal scale ranging from 0 (not at all) to 6 (every day)

This scale was used in the brain bank samples obtained from London.

2.2.1.4 Columbia University Scale to Assess Psychopathology in Alzheimer's disease (CUSPAD)

The CUSPAD tool is a brief assessment of symptoms of psychosis, behavioural disturbance and depression in AD (Devanand, Miller et al. 1992). Assessment is administered by an investigator and considers the symptoms over the four weeks preceding the interview. For psychotic symptoms a general question about the presence of delusions or hallucinations is followed up by more specific questions designed to ascertain their exact nature (e.g. auditory or visual hallucinations/paranoia or misidentifications) in a decision tree format. Symptoms are categorised as present or absent.

2.2.2 Determining the presence of psychotic symptoms

Several different rating scales were used in both AD and DLB/PDD cohorts. As such, symptom scores at each visit were converted into a binary present/absent rating according to the rules set out in Table 2-2.

Table 2-2 Rules for the classification of delusions and hallucinations for each rating scale

Rating Scale	Score used	Symptom present	Symptom absent
NPI	Delusions, hallucinations: frequency x severity	Score >0	Score = 0
CAMDEX	Hallucinations, persecutory ideas	Symptom score = 1	Symptom score = 0
PBE	Hallucinations, delusions defined as positive rating on grandiose ideas or persecutory ideas items. Frequency scores used for both	Symptom frequency score >0 (for delusions either of the items can be rated >0)	Symptom frequency score = 0 (for delusions all of the items must be rated 0)
CUSPAD	First general question relating to the presence/absence of symptoms	Symptom score = 1	Symptom score = 0

2.2.3 Cognitive impairment and disease severity

2.2.3.1 Mini Mental State Examination (MMSE)

The MMSE (Folstein, Folstein et al. 1975) is a 30 point questionnaire designed to assess cognitive impairment in dementia. It is widely used both clinically and in research. The question assesses six cognitive domains: 1) orientation (to time and place); 2) registration (repeating the names of three objects until remembered); 3) attention/calculation (either subtracting 7 from 100 then from 93 and so on or spelling "world" backwards); 4) recall (of the words in the registration section); 5) language (naming objects, repetition of a phrase, following an instruction, writing a sentence) and, 6) the copying of a complex shape.

The MMSE is not used as the sole means of clinically diagnosing dementia but a total score of 27-30 would be consistent with a cognitively normal individual while a score below that may indicate dementia. In research, the MMSE is frequently used as an indicator for dementia severity, with a score of 20 and above, 10-19 and 0-9 indicating mild, moderate and severe dementia respectively.

2.2.3.2 Functional Assessment Staging Tool (FAST)

The MMSE is based purely on cognition, another way of staging an individual's dementia severity is through the assessment of functional/activities of daily living (ADL) impairment. The FAST (Reisberg, Ferris et al. 1985) is designed to assess just this. It is an ordinal scale ranging from 1 (normal aging) through 7 (late stage dementia) and to be scored deficits must be related to the dementia and not another medical condition, such as stroke or physical disability. Stage 4 represents mild dementia and is given when an individual's ADL becomes affected (e.g. cooking, cleaning, finances). In stage 5 individuals would have problems selecting the correct clothing. Stage 6 is split into five sub-sections (6a to 6e): in 6a an individual would need help putting on clothes while b, c, d and e represent further more serious impairments (bathing, toileting and urinary and faecal incontinence). Similarly stage 7 is split into 7a through 7f. These are the last stages of dementia where an individual's speech is limited to a few or a single word(s) per day (7a

and b) and subsequently they can no longer walk, sit up, smile or hold their head up (7c, d, e and f).

2.3 Medication history

The protocols for each study included the recording of current medication at each visit, although dose was not consistently recorded either between or sometimes within studies. Medication history for the ARUK cohort was manually recorded for each visit from the paper notes of all patients and transferred to Excel. For datasets which already included medication history, this was not universally coded and often existed in list format rather than each drug occupying its own cell in Excel. A list of psychotropic drugs was created and broadly categorised into antipsychotics (each drug had its own code), antidepressants, sedatives, anti-dementia drugs and histamine H1 antagonists. Medication doses were not consistently available from all cohorts therefore this information was not collected. I wrote Excel formulae to search lists for the names of relevant drugs and recode into numbers.

2.4 DNA sources and preparation

2.4.1 Buccal mucosa sample collection and DNA preparation

Buccal mucosa cells were collected from individuals enrolled in the following cohorts: MAIN-AD, MAGD, Norway and WHELD. Collection kits (10 cotton wool buds and a 15ml plastic tube (Sarstedt Ltd.) containing 2.5ml collection buffer (see below) were provided by the Social, Genetic and Developmental Psychiatry (SGDP) Centre at the Institute of Psychiatry, King's College London, as part of a service they operate which also involves DNA extraction, quality testing, determination of concentration and storage (Freeman, Smith et al. 2003).

Buccal mucosa cells were removed by rubbing cotton wool buds on the inside of the mouth (i.e. cheeks and lips). Normally a person can do this themselves, however for people with dementia it was much more effective for the research team to conduct the procedure, due to the impaired comprehension and language abilities in many of these individuals. Where possible the research

teams of the various studies had face to face training provided by Byron Creese, however in most cases training was provided over the phone with an accompanying video and step-by-step instruction sheet.

Up to ten buds are used per participant, with a greater number of buds yielding a greater amount of DNA. Each bud is rubbed on a different part of the participant's mouth for approximately 20 seconds. After use, each bud is placed in the plastic tube so that one plastic tube is used for all the buds collected from a participant. The buffer consists of the following: 100 mM NaCl, 10 mM Tris-HCl pH 8.0, 10 mM ethylene diaminetetraacetic acid (EDTA) pH 8.0 with 0.2 mg/ml proteinease potassium and 0.5% w/v sodium dodecyl sulphate.

Samples were then sent to the SGDP for DNA extraction (according to an established protocol: Freeman et al., 2003) and storage until requested for use.

2.4.2 DNA from blood and brain samples

DNA from blood and brain was obtained from participants in the following cohorts: ARUK, ANM, DCR, DEMVEST, OPTIMA, Greek, brain banks and non dementia controls. The DNA for all was extracted and stored by the research teams at the respective institutions responsible for each cohort and samples were requested for use for this project, with the exception of DEMVEST. Here, blood was extracted locally and sent to KCL (Wolfson Centre for Age-Related Diseases) for extraction (by Dr Emma Jones) and storage since 2008.

2.5 SNP selection

The experiments that follow used a candidate gene approach. Resources and time dictated that only a small number of SNPs could be investigated in this thesis. There are around 20,000 genes in the human genome and many millions of SNPs therefore picking a SNP at random will have such a small likelihood of being associated with a given phenotype as to make investigation worthless. Before detailing genotyping methods the process for selecting which SNPs to examine will be discussed. In selecting SNPs we used the following criteria:

- Biological plausibility: SNPs had to be in psychosis-relevant pathways.
- Support from *in vivo* or *in vitro* models: SNPs had to have known functional consequences (e.g. they lead to an amino acid change or are transcription altering).
- Prior evidence of genetic association.
- Common enough in European samples to warrant investigation in cohorts of the size used in these experiments.

On the basis of the above criteria the following four polymorphisms were investigated: 5HTTLPR, *COMT* val158met, extended *MAPT* haplotype and *HNMT.* A full review of the functional significance and supporting evidence for the selection of these polymorphisms is can be found in sections 1.4.1 (5HTTLPR), 1.4.2 (*COMT* val158met), 1.4.3 (extended *MAPT* haplotype) and 1.4.6.1 (*HNMT).*

It is acknowledged that there are a number of other commonly studied polymorphisms. Most notably among the serotonin acting polymorphisms is $5HT_{2A}$. Although this is a well studied polymorphism in AD psychosis and in a biological plausible pathway it is synonymous and it is not clear how it would exert its effect on psychotic symptoms if such an effect was found. Therefore 5HTTLPR was prioritised in this study; it is as well studied as $5HT_{2A}$ but there is a far greater body of evidence to support its role in the pathogenesis of psychotic symptoms.

2.6 Genotyping

I carried out the genotyping blind to symptom status. Taqman SNP genotyping assays were used to analyse the *COMT* val158met and *HNMT* polymorphism while electrophoresis or a custom Taqman assay was used to analyse the 5HTTLPR polymorphism. The *MAPT* haplotype was also analysed by electrophoresis. Each procedure is described in detail below.

2.6.1 Assay to Detect the val158met Polymorphism in *COMT* and the thr105ile Polymorphism in *HNMT*

2.5 ng DNA from each sample was pipetted onto a 96- or 384-well reaction plate (Applied Biosystems) to be included in a reaction mixture with a total volume of 25 µl (for 96-well plates)

also containing 1x TaqMan SNP Genotyping assay (Applied Biosystems, containing forward and reverse primers and two allele specific probes, see below) and 1x TaqMan Universal PCR Master Mix (AmpliTaq Gold DNA polymerase, AmpErase UNG, dNTPs with dUTP, passive reference, and buffer). In the case of 384-well plates the total reaction volume was 5 µl, with the volumes of assay and mastermix adjusted proportionally. One non-template control (NTC) was included one each plate.

Each TaqMan SNP Genotyping assay contains two allele specific probes 5'-labelled with VIC or FAM reporter dyes and 3'-labelled with a non-fluorescent quencher, which when in close proximity to the reporter dyes inhibits their fluorescence. During PCR the probes bind to the site of their respective allele. During the DNA synthesis stage, AmpliTaq Gold DNA polymerase cleaves the dye from the probe, allowing it to fluoresce. End point fluorescence was read using an ABI Prism 7000 or 7900 Sequence Detection System. In individuals homozygous for allele-1 or allele-2 only VIC or FAM fluorescence respectively is detected, whilst in heterozygotes both VIC and FAM dyes is detected (see Figure 2-1).

Genotyping of the *COMT* val158met polymorphism in the ARUK and Greek cohorts was carried out by the lab of Dr John Powell, King's College London and data were made available for this study. I carried out the *COMT* val158met genotyping for all the remaining samples.

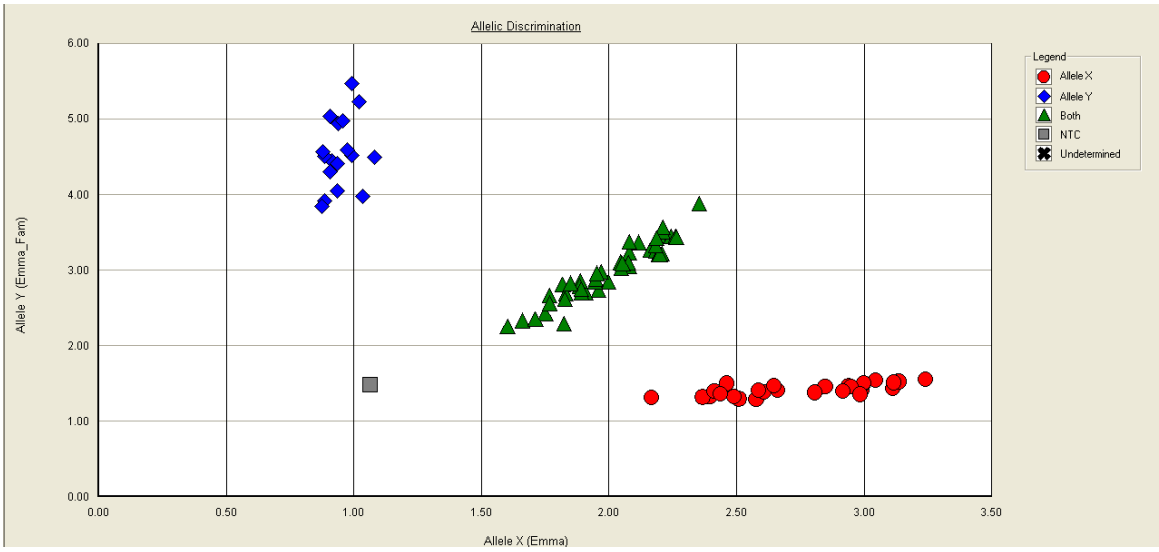

Figure 2-1 ABI SDS example output for COMT val158met and HNMT polymorphisms

Three fluorescence clusters are shown which correspond to the three possible genotypes: blue and red=homozygous, green=heterozygous (specific calls would depend on the polymorphism but the principal is exactly the same). Grey square=NTC

2.6.2 Assay to Detect the 5HTTLPR Polymorphism in *SLC6A4*

I used two methods to detect the 5HTTLPR polymorphism, the first was PCR followed by electrophoresis and the second was a custom Taqman SNP genotyping assay (Applied Biosystems) used because of the need for a high throughput method.

Genotyping of the 5HTTLPR polymorphism in the ARUK and Greek cohorts was carried out by the lab of Dr John Powell, King's College London and data were made available for this study. I carried out the 5HTTLPR genotyping for all the remaining samples.

2.6.2.1 Electrophoresis

2.6.2.1.1 5HTTLPR deletion

25 ng DNA, 400 nM of primers 5'-GAGGGACTGAGCTGGACAACCAC-3' and 5'-GGCGTTGCCGCTCTGAATGC-3' (Sigma-Aldrich), 1x Q solution, 1x PCR buffer, 2 units of Taq DNA polymerase and 200 µM dNTPs (all from Qiagen) were included in a total reaction volume of 50 µl. The following cycling conditions were used: 95°C for 1 minute, 40 cycles of (95°C for 30 seconds, 61°C for 30 seconds, 72°C for 1 minute), 72°C for 10 minutes. PCR products were analysed by electrophoresis on a 2% agarose gel. The S allele is represented as a band of 484bp and the L allele as a band of 528bp (Figure 2-2).

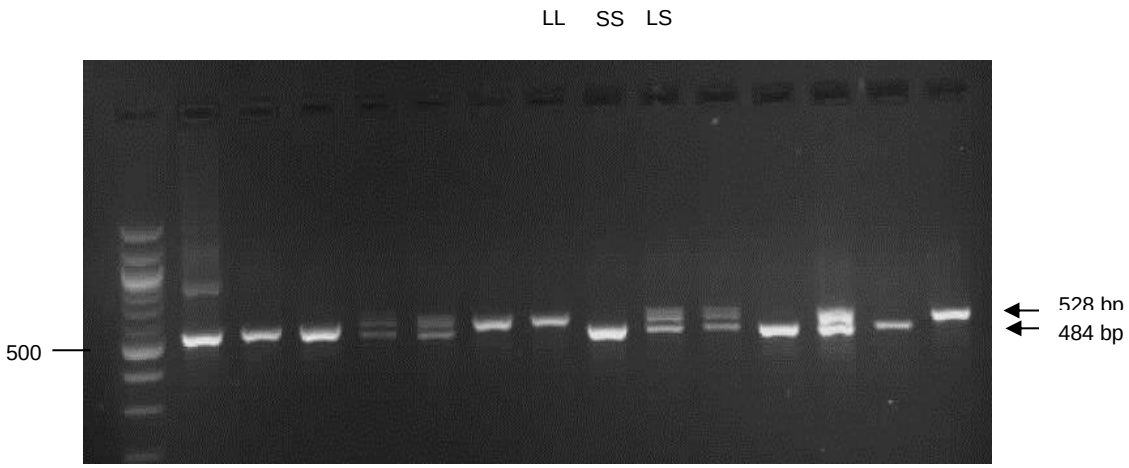

Figure 2-2 A 2% agarose gel image of the 5HTTLPR polymorphism

Far left lane: 100 bp DNA ladder. A 528 bp band represents LL genotype while a smaller 484 bp (i.e. samples with the 44 bp deletion) band represents the SS genotype. Heterozygotes are therefore represented by two bands, one of each size

I used this method on the DLB/PDD cases and on the POCD controls. Genotyping of the DLB/PDD cases was carried out by Dr Emma Jones.

2.6.2.1.2 5HTT rs25531 SNP

Genotyping of the rs25531 SNP, the G allele of which is almost always inherited with the L allele and reduces transcriptional activity to that of the S allele, was also attempted. The protocol proposed by Wendland et al. (2006) was followed. Broadly it specifies a method similar to the above but with an additional digest step before electrophoresis (Wendland, Martin et al. 2006). 200nM of forward (5'-TCCTCCGCTTTGGCGCCTCTTCC-3') and reverse (5'-TGGGGGTTGCAGGGGAGATCCTG-3') primers were included along with the reagents specified in the section above at the same concentrations and 5 ng of DNA in a total reaction volume of 20 µl. Following PCR cycling (95ºC for 15 minutes, followed by 35 cycles of (94ºC for 30 seconds, 65.5 ºC for 90 seconds, 72 ºC for 60 seconds), 72ºC for 10 minutes) 7 µl of product was digested with 1x buffer and *HpaII* (New England Biolabs) in a total volume of 20 µl for 3 hours. The G allele of rs25531 creates a restriction site recognised by *HpaII* which cuts the DNA fragment creating two bands, whereas fragments carrying the more common A allele are not digested. Lastly, 4 µl of the restriction digest and 18 µl of the original PCR product were loaded onto a 3% agarose gel.

This assay was first tested on DNA from several different sources but the bands after digestion were too faint to make a reliable genotype call. In order to address this problem the assay was optimised by first removing Q solution and then by adding 0, 1, 2 and 3 µl of $MgCl_2$, as suggested by the manufacturers (Qiagen). However in no instance did this improve the quality of the DNA bands visualised by electrophoresis. Due to time constraints at this stage of the project and the need to process a large number of samples a high throughput method of genotyping 5HTTLPR was opted for and the genotyping of rs25531 was postponed.

2.6.2.2 Custom Taqman SNP genotyping assay to detect the 5HTTLPR polymorphism

This assay was adapted from Covault et al. (Covault, Tennen et al. 2007). 25 ng of DNA was pipetted into a 96-well reaction plate (Applied Biosystems) for inclusion in a total reaction volume of 30 µl. The remaining 25 µl volume of reaction mixture consisted of 200nM of each forward and reverse primer (5'-GCAACCTCCCAGCAACTCCCTGTA-3' and 5'-GAGGTGCAGGGGGATGCTGGAA-3' respectively) and 60nM and 120nM of VIC and one FAM labelled probes respectively (all obtained from Applied Biosystems constructed as described in section 2.6.1), 1x Universal PCR Master mix and 1x Q solution. The FAM labelled probe (TGCAGCCCCCCCAGCATCTCCC) is specific to the L allele as it binds to a section of DNA within the 44 bp deletion. The target sequence of the VIC probe (TCCCCCCCTTCACCCCTCGCGGCATCC) is present in both the L and S alleles and serves as a positive internal control. One NTC was included on each plate along with three samples (one of each genotype) for which genotype had been verified by electrophoresis.

The following cycling conditions were used: 50°C for 2 minutes, 95°C for 10 minutes then 50 cycles of (98°C for 15 seconds, 62.5°C for 90 seconds).

The three genotypes (LL, LS and SS) are differentiated by the level of FAM fluorescence detectable. The authors (Covault et al., University of Connecticut) were contacted and agreed to provide their raw ABI SDS output, shown Figure 2-3 with the raw data from this study in shown in Figure 2-4.

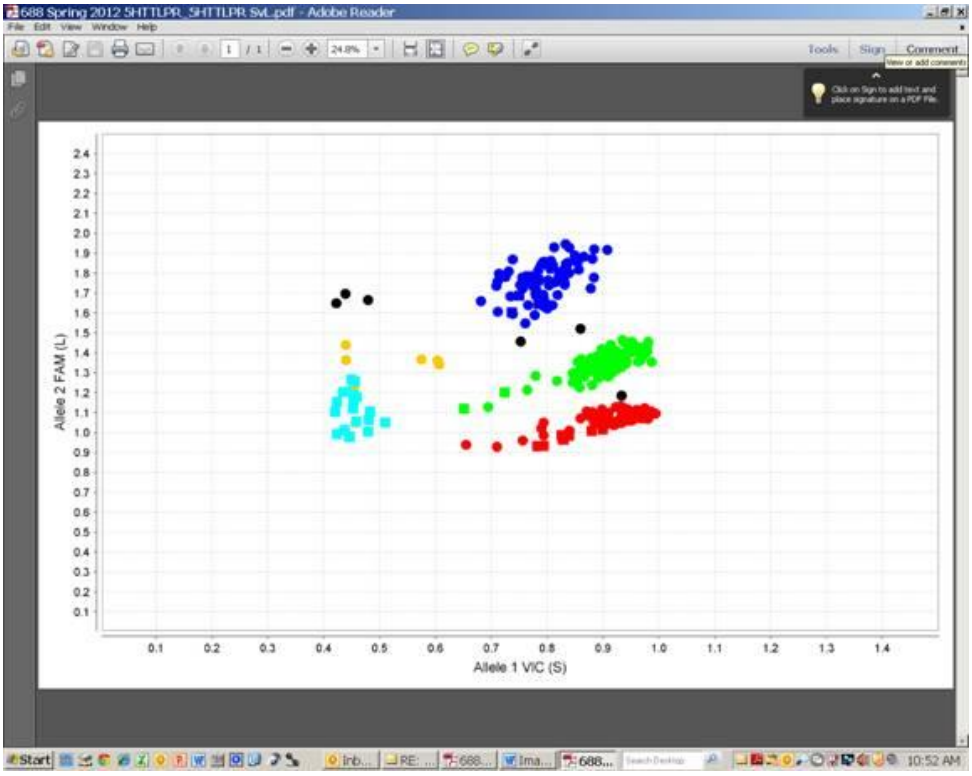

Figure 2-3 SDS output provided by Dr Jonathan Covault and Christine Abreu (University of Connecticut)

Coloured clusters as per A; black dot=undetermined; turquoise dot=NTC; yellow dot=non amplifiers

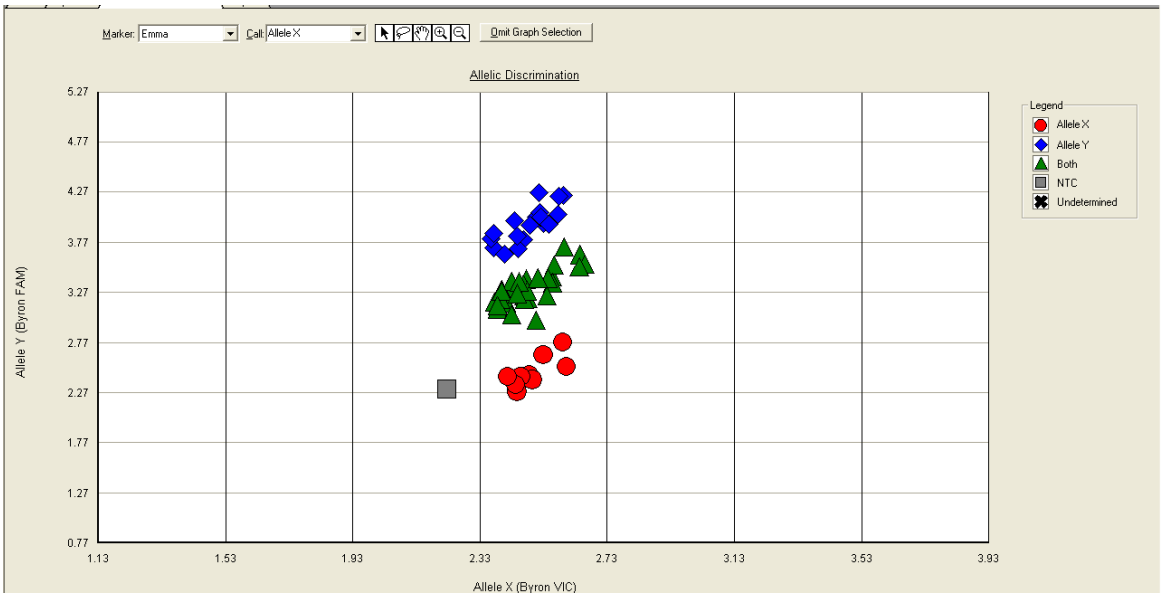

Figure 2-4 SDS 7000HT output showing three genotype clusters of the 5HTTLPR polymorphism

The VIC labelled probe (x axis) fluoresces at the same wavelength for all cases because its target sequence is in the amplicon of both alleles. The FAM fluorescence (y axis) detected is dependent on the number of L allele present as the probe's target sequence is contained in the deleted 44 bp sequence: red=0 (SS genotype), green=1 (LS genotype), blue=2 (LL genotype); grey square=NTC; black cross=undetermined.

2.6.3 Assay to detect the extended *MAPT* haplotype

25 ng DNA along with 100 nM of primers 5' -GGAAGACGTTCTCACTGATCTG-3' and 5 '-AGGAGTCTGGCTTCAGTCTCTCTC-3', 200 µM dNTPs, 1.25 units Taq DNA polymerase and 1x PCR buffer were included in a total reaction volume of 50 µl. A touchdown PCR method was used to optimise target sequence amplification. The starting temperature of 58°C was decreased by 0.2 each of the 35 cycles using the following conditions: (94°C for 30 seconds, touchdown annealing for 30 seconds, 72°C for 30 seconds). Amplified DNA fragments were analysed by electrophoresis and visualised on a 1% agarose gel.

H1/H2 haplotype discrimination is based on the presence/absence of a 238bp deletion in intron 9 which is found only in the H2 haplotype (Baker, Litvan et al. 1999). H1 homozygotes are represented by a 484 bp band while H2 homozygotes are represented by a 246 bp band (Figure 2-5).

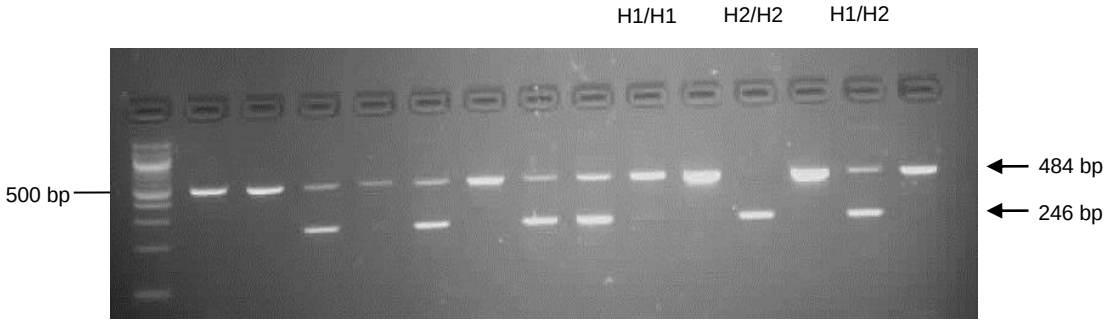

Figure 2-5 A 1% agarose gel image of the extended tau haplotype
Far left lane: 100 bp DNA ladder. A 484 bp band represents H1/H1 haplotype while a smaller 246 bp (i.e. samples with the 238 bp deletion) band represents the H2/H2 haplotype. Heterozygotes are represented by a band of each size

2.7 Statistical analyses

Analysis plans for each study are described in detail in their respective results chapters. Briefly, outcomes were either categorical or continuous level and in the case of the latter datasets were examined for deviations from parametric data assumptions and appropriate methods were employed accordingly. The analysis presented in Chapter 6 was carried out under the guidance

of Dr Chin-Kuo Chang, South London and the Maudsley Biomedical Research Centre Nucleus. Power calculations were performed using QUANTO (Gauderman, Morrison 2009).

Chapter 3 Determining the association of 5HTTLPR and *COMT* polymorphisms with delusions and hallucinations in AD and DLB/PDD

Related papers:

- Creese, B., Ballard, C., Aarsland, D., Londos, E., Sharp, S., Jones, E. (In Press) Determining the Association of the 5HTTLPR Polymorphism with Delusions and Hallucinations in Lewy Body Dementias. *Am J Geriatr Psych.*
- Creese, B., Ballard, C., Aarsland, D., Londos, E., Sharp, S., Jones, E. (2013) No Association of COMT val158met Polymorphism in Lewy Body Dementias. *Neurosci Lett.* 531(1), 1-4.

3.1 Summary introduction

Neuropsychiatric symptoms are common in all forms of dementia and psychosis is of particular importance in people with DLB/PDD and AD, with more than 70% of patients experiencing visual hallucinations and more than 60% of patients experiencing delusions in the former. While the frequency is less in AD (at around 30-50%) both symptoms are highly persistent and distressing in a significant proportion of individuals (Ballard, O'Brien et al. 2001).

Clinical correlates of psychotic symptoms include sensory deprivation, diminished consciousness, early visuospatial deficits and cognitive impairment (McKeith, Galasko et al. 1996, Hamilton, Landy et al. 2011), all of which highlight the importance of longitudinal evaluations in accurately distinguishing transient and persistent psychotic symptoms and to inform clinical treatment decisions. The association of the severity of cognitive impairment and psychosis is less consistent in DLB/PDD than in AD (Ballard, Holmes et al. 1999, Del Ser, McKeith et al. 2000, Aarsland, Cummings et al. 2001), but remains an important potential confounder which needs to be considered in the design and analysis of studies examining the biological associations of psychosis in these individuals.

Despite the prevalence, persistence and distress caused by psychotic symptoms, safe and effective treatment options are limited. There is RCT evidence of a modest improvement of visual hallucinations with the cholinesterase inhibitor rivastigmine in patients with DLB and PDD (Wesnes, McKeith et al. 2002, Emre, Aarsland et al. 2004) and although open trials of antipsychotics have suggested some benefit (The Parkinson Study Group 1999), the risk of severe neuroleptic sensitivity reactions is substantial in these individuals (McKeith, Fairbairn et al. 1992). In AD there is evidence of a modest effect of risperidone, olanzapine and aripiprazole however there are safety concerns surrounding these drugs and other antipsychotics which are still commonly used (Ballard, Howard 2006). There is therefore an urgent need to identify safer and more effective therapies for psychosis in people with all three types of dementia, which has to be based upon a better understanding of the underlying distinct pathological mechanisms as well as those which may be shared with the same symptoms across different dementias. In the absence of effective animal models of psychosis in dementia, post-mortem, imaging and genetic studies will be vital in achieving this mechanistic understanding.

Post-mortem studies have highlighted the serotonergic system as an important correlate of psychotic symptoms and other neuropsychiatric symptoms in AD, DLB and PDD (Lanctot, Herrmann et al. 2001). Cheng et al. (1991) reported preserved $5HT_{2A}$ binding in DLB patients with hallucinations compared to those without, while Perry et al. (1990) cite a monoaminergic-cholinergic imbalance as an important neurochemical correlate (Cheng, Ferrier et al. 1991, Perry, Marshall et al. 1990). There is limited evidence specifically focusing on serotonergic associations of hallucinations in PDD, however in PD psychosis two recent binding studies have reported greater $5HT_{2A}$ binding in patients with hallucinations than those without (Huot et al., 2010 and Ballanger et al, 2010), paralleling previous reports in people with DLB. There is also evidence of neurochemical changes associated specifically with delusions in DLB patients (Ballard, Piggott et al. 2000). Here the authors found elevated muscarinic M1 receptor binding to be associated with delusions but not hallucinations thus indicating there may be distinct mechanisms underlying these two symptoms of psychosis.

Genetic studies in DLB and PDD are lacking but in the wider AD literature there have been a number of reported associations between polymorphisms in serotonergic pathway genes and psychosis (DeMichele-Sweet, Sweet 2010, Ballard, Howard 2006). Although there are inconsistencies, the strongest evidence is for the 5HTTLPR polymorphism. Studies by Sweet et al. (2001) and Quaranta et al. (2009) in AD have both reported a positive association between the L allele and LL genotype and psychotic symptoms (Quaranta, Bizzarro et al. 2009, Sweet, Pollock et al. 2001), although this is not a consistent finding (Proitsi, Lupton et al. 2010, Borroni, Grassi et al. 2006). Conversely, the only study in PD psychosis failed to find a significant association with the $5HT_{2A}$ T102C receptor polymorphism or 5HTTLPR and visual hallucinations (Kiferle, Ceravolo et al. 2007). In PDD and DLB there have been no such studies to date.

Similarly, there is evidence from *in vivo* PET imaging of dopaminergic correlates of psychotic symptoms in AD (Reeves, Brown et al. 2009). Of those polymorphisms investigated in AD, the only one to also have been studied in DLB is *COMT* val158met in the catechol-O-methyl transferase (*COMT*) gene. Some, but not all (Sweet, Devlin et al. 2005, Proitsi, Lupton et al. 2010, Borroni, Agosti et al. 2004), previous studies in AD have implicated the *COMT* gene as a candidate for psychosis based on an association with *COMT* val158met, while in DLB/PDD there

has been just one previous study which, like those in AD, reported an association with the high activity val allele and psychosis (Borroni, Di Luca et al. 2006). More broadly in Lewy body disease, one study in PD psychosis found no association with *COMT* val158met (Camicioli, Rajput et al. 2005). These findings collectively suggest that there may be shared mechanisms underlying psychotic symptoms in AD and DLB/PDD. Establishing such mechanisms would have important implications for treatment approaches in dementia, however further research is urgently required to confirm this.

Finally, it has been proposed that, because of the faster rate of cognitive decline that accompanies psychotic symptoms, the high heritability and the higher burden of NFT pathology, AD with psychotic symptoms represents a phenotype of AD distinct from patients who present without psychosis (Sweet, Nimgaonkar et al. 2003). It is hypothesised there are genes that specifically contribute to AD with psychotic symptoms (the heterogeneity model) and those that only contribute to psychosis after the neurodegeneration of AD has set in (disease modifier model). Testing to which scenario a particular polymorphism contributes requires the addition of a non-dementia control group with which to compare genotype frequencies in patients with symptoms. This study will therefore evaluate both models in AD and DLB/PDD. However, a significant finding is not expected for the heterogeneity model because there is no evidence to suggest that 5HTTLPR or *COMT* val158met are involved in AD or DLB/PDD risk.

This study examines the relationship between 5HTTLPR and *COMT* val158met and persistent delusions and hallucinations in two cohorts of patients with DLB/PDD and AD. It will also explore the nature of these polymorphisms' contributions to psychotic symptoms by evaluating them in disease modifier and heterogeneity contexts as described above.

3.2 Hypotheses

- Patients with the LL genotype will carry an increased risk of both delusions and hallucinations in DLB/PDD and AD.
- The *COMT* val/val genotype will be associated with the presence of psychotic symptoms in DLB/PDD and AD.

- There will be no support for the heterogeneity model of psychotic symptoms in either AD or DLB/PDD.

3.3 Methods

3.3.1 Software

STATA/SPSS: data analysis and coding.

3.3.2 Inclusion/exclusion criteria

Inclusion criteria:

- A diagnosis of probable, possible or definite AD according to the NINCDS-ADRDA criteria (McKhann, Drachman et al. 1984) or;
- A diagnosis of DLB/PDD according to the McKeith et al. (2005) consensus criteria (McKeith, Dickson et al. 2005) or;
- Queen Square criteria for PD (Hughes, Daniel et al. 1992) and DSM-IV criteria for dementia occurring >1 year after the onset of PD symptoms.
- At least two assessments of delusions and/or hallucinations six months or more apart.
- MMSE or other measure of dementia severity at last assessment.

Exclusion criteria:

- History of a psychotic disorder not related to dementia.

3.3.3 Design and cohort

The DLB/PDD patients were drawn from the brain bank and DEMVEST cohorts. By definition all 21 of the brain bank patients were suitable for this study while 80 from the DEMVEST cohort also met the criteria. The AD patients were drawn from the MAIN-AD, OPTIMA, Norway, WHELD, DEMVEST, Greek, ARUK, DCR and ANM cohorts. The 111 cognitively normal controls from the

ANM and POCD cohorts were included as a comparison group for the AD study and 92 controls from the POCD cohort served as the comparison group for the DLB/PDD study.

See sections 2.6.1 and 2.6.2 for genotyping methods.

3.3.4 Classification of symptoms

For the AD patients the Cambridge Mental Disorders of the Elderly Examination (CAMDEX) semi-structured assessment was used to assess psychotic symptoms in 87 individuals while the Neuropsychiatric Inventory (NPI) was used in the remaining 487. Those from the DLB/PDD cohort were either assessed with the Present Behavioural Examination (PBE)/Columbia University Scale to Assess Psychopathology in AD (CUSPAD) (brain banks) or NPI (DEMVEST). Because of the different rating scales used a binary present/absent approach to symptom classification was adopted (see section 2.2.2).

Patients were first either rated as having persistent, transient or no symptoms. Symptoms were considered persistent if they were present for more than six months continuously or recurred more than six months after resolution. Being 'present' at a visit was in this case defined as a score >0 on any of the assessment scales. Any individuals with transient symptoms, that is those with symptoms not falling under the definition of persistent, were classified as not having the symptom. The diagnostic criteria for AD psychosis set out by Jeste and Finkel (Jeste, Finkel 2000) stipulates that symptoms must occur, at least intermittently, for 1 month or longer and not occur exclusively during delirium. In this study as one month follow-up data was not universally available and as there were no measurements specifically for delirium it was considered prudent to employ the six month threshold for persistent symptoms in order to exclude these episodes of delirium from the hallucination and delusion groups. This threshold is supported by a recent study which indicated that subsyndromal delirium can be persistent but usually resolves within six months from baseline (Cole, McCusker et al. 2012).

3.3.5 Other data

Demographic data, cognitive evaluations with the Mini Mental State Examination (MMSE) at the last assessment and the presence of PD (based upon the Unified Parkinson's Disease Rating Scale or the Hoehn and Yahr staging system and applying only to the DLB/PDD cohort) were also recorded.

For the AD samples exposure to antipsychotics and anti-dementia drugs was coded as either 'ever' during the period of follow up or 'never.'

3.3.6 Statistical analysis

The analyses that follow are split by disease but follow the same plan outlined below (except where noted). Section 3.4 examines DLB/PDD patients and section 3.5 AD patients.

The frequency of dementia with delusions versus dementia without delusions and dementia with hallucinations versus dementia without hallucinations was evaluated in two separate binary logistic regression models for each polymorphism. Two explanatory variables were included in each model: genotype as a categorical variable with three levels (LL, LS and SS or val/val, val/met and met/met) and MMSE score as a continuous variable (or for the AD analysis disease severity was treated as another categorical variable with three levels: mild, moderate and severe). On the basis of previous data in AD the SS genotype was used as the reference for 5HTTLPR genotype and the met/met as the reference for the *COMT* analysis. Analysis of symptom frequency by allele was also conducted.

The cognitively normal controls were included in a subsequent analysis. Four further regression models were run analysing those with delusions versus controls, those without delusions versus controls and likewise for hallucinations. The 5HTTLPR or *COMT* val158met polymorphism was the single explanatory variable in these models and was treated in the same way as above.

A final secondary analysis explored the use of 6 months threshold for persistent symptoms. In this analysis, patients classified as having only transient symptoms were excluded, leaving those with persistent delusions analysed again those with no delusions at all, and likewise for hallucinations.

The significance threshold was set at 0.01.

3.3.6.1 Power calculation

In all cases genotypes were examined in an additive model. For DLB/PDD assuming minor allele frequency (MAF) of 0.45 and a symptom frequency of 45% this sample size would have 80% power to detect an effect size (expressed as odd ratio, OR) of 2.4 associated with the risk allele and 50% power for OR 1.8

For AD, a lower frequency of symptoms would be expected. Therefore, assuming a symptom frequency of 20% and the remaining parameters to be otherwise as above the AD sample would have 80% power to detect OR 1.5 and 44% to detect OR 1.3.

3.4 Results: Lewy body dementias

3.4.1 Descriptive statistics

Of the 101 DLB/PDD patients, 11 were confirmed at autopsy. Ninety-eight were successfully genotyped for the 5HTTLPR and *COMT* val158met polymorphism. The three that were unsuccessfully genotyped were different for each polymorphism therefore in the analysis that follows they are presented separately. One patient had data for delusions but not hallucinations and one patient had data for hallucinations but not delusions. The remainder of the patients had data for both symptoms (n=96). Prospective data regarding persistent delusions and hallucinations were available for all of these individuals. Eighty-seven had PDD and 10 had DLB.

Ninety-two controls from the POCD cohort were used as a comparison group, they did not differ in terms of gender frequency (70% female: χ^2=0.35, df=1, p=0.9) or age (mean age 77: U=-0.1, df=185, p=0.06). Two samples could not be successfully genotyped for 5HTTLPR, leaving 90 in this analysis.

Genotype frequencies for 5HTTLPR and *COMT* val158met did not deviate significantly from Hardy-Weinberg equilibrium (χ^2=0. 3, p>0.05; χ^2=0.1, p>0.05 respectively). Descriptive statistics by 5HTTLPR and *COMT* val158met polymorphism are shown in Table 3-1 and

		5HTTLPR [Mean±SD/ Frequency(%)]		
		SS (n=21)	**LS (n=46)**	**LL (n=31)**
Age[1]		74 (5.4)	78 (6.8)	75 (5.9)
Gender[3]	Female	12 (57)	29 (63)	14 (45)
	Male	9 (43)	17 (37)	17 (55)
MMSE[3]		19 (7.9)	18 (7.9)	21 (5.6)

1. *F*(2,94)=2.3, p=0.1;**2.** χ^2=2.4, df=2, p=0.3; **3.** χ^2=2.4, df=2, p=0.3

Table 3-2 respectively (N=98 because the two patients missing delusions or hallucinations were both included): there were no differences in frequencies or means for age (5HTTLPR: *F*(2,94)=2.3, p=0.1; *COMT* val158met: *F*(2,94)=0.4, p=0.7), gender (5HTTLPR: χ^2=2.4, df=2, p=0.3; *COMT* val158met: χ^2=0.6, df=2, p=0.7) or MMSE (Kruskal-Wallis test, 5HTTLPR: χ^2=2.4, df=2, p=0.3; *COMT* val158met: χ^2=2.7, df=2, p=0.3) by genotype.

Table 3-1 Participant demographics by 5HTTLPR genotype (N=98)

		5HTTLPR [Mean±SD/ Frequency(%)]		
		SS (n=21)	**LS (n=46)**	**LL (n=31)**
Age[1]		74 (5.4)	78 (6.8)	75 (5.9)
Gender[3]	Female	12 (57)	29 (63)	14 (45)
	Male	9 (43)	17 (37)	17 (55)
MMSE[3]		19 (7.9)	18 (7.9)	21 (5.6)

1. $F(2,94)=2.3$, p=0.1;**2.** $\chi^2=2.4$, df=2, p=0.3; **3.** $\chi^2=2.4$, df=2, p=0.3

Table 3-2 Participant demographics by COMT val158met genotype (N=98)

		COMT val158met [Mean±SD/ Frequency(%)]		
		val/val (n=21)	**Val/met (n=50)**	**met/met (n=27)**
Age[1]		76 (5.8)	77 (5.7)	76 (7.1)
Gender[2]	Female	11 (52)	30 (60)	14 (52)
	Male	10 (48)	20 (40)	13 (48)
MMSE[3]		17 (8.3)	19 (7.3)	20 (6.4)

1. $F(2,94)=0.4$, p=0.7; **2.** $\chi^2=0.6$, df=2, p=0.7; **3.** $\chi^2=2.7$, df=2, p=0.3

The primary analyses for the DLB/PDD patients below are organised by genotype, starting with 5HTTLPR and followed by *COMT* val158met.

3.4.2 Primary analysis: 5HTTLPR

Twenty-nine (30%) individuals had persistent delusions and of the 68 (70%) classified has having no delusions 20 (29%) had non-persistent delusions. These groups were termed the delusions group and no delusions group respectively. Most of those with delusions (75%) and 24 (35%) of those with no delusions also had hallucinations. MMSE among those with persistent delusions was significantly lower than those without (t=4.3, df=95, p<0.001). There were no differences in age (t=-0.5, df=94, p=0.6) or gender ($\chi^2 = 0.4$, df=1, p=0.8) between these groups (Table 3-3, page 111).

Hallucinations were the most common symptom with 46 individuals (47%) classified as having persistent symptoms, henceforth known as the hallucination group. Of the 51 (53%) people classified as having no hallucinations, 20 (41%) had non-persistent hallucinations. Among those with hallucinations 22 (48%) also had delusions, while in the no hallucinations group 7 (14%) had delusions. There were no differences in mean age (t=-0.3, df=94, p=0.74), mean MMSE (t=1, df=95, p=0.3) or gender (χ^2 = 1.1, df=1, p=0.3) between those with and without hallucinations (Table 3-4, page 111).

3.4.2.1 Univariate analysis: 5HTTLPR

Univariate analysis was conducted first (Table 3-3). For delusions, there was a trend for LL genotypes to carry an increased risk for delusions but this did not meet the more stringent 0.01 alpha level used for this study. For every one point decrease in MMSE an individual's odds of having persistent delusions were increased by 1.1 (p<0.001). Analysis by allele indicated a non-statistically significant trend effect of the L allele for persistent delusions (OR=2, 95% CI=1.05-3.8, p=0.03).

Table 3-3 Logistic regression: persistent delusions in DLB/PDD (N=97)

Risk Factor		Persistent delusions [Mean ±SD / Frequency (%)]		Odd ratio (95% CI)	p-value
		No (n=68)	Yes (n=29)		
Age		76 (5.9)	76 (7)	1 (0.9-1.1)	0.6
Gender	Female	39 (57)	16 (55)	Reference	-
	Male	29 (43)	13 (45)	1.1 (0.5-2.6)	0.8
MMSE		21 (6.1)	15 (7.5)	0.9 (0.8-0.9)	<0.001
5HTTLPR	SS	18 (26)	2 (7)	Reference	-
	LS	31 (46)	15 (52)	4.3 (0.8-21.2)	0.07
	LL	19 (28)	12 (41)	5.6 (1.1-29)	0.04
5HTTLPR allele	S	67 (49)	19 (33)	Reference	-
	L	69 (51)	39 (67)	2 (1.05-3.8)	0.03

In univariate analysis for hallucinations none of the variables presented in Table 3-4 emerged as a significant risk factor for persistent hallucinations.

Table 3-4 Logistic regression: persistent hallucinations in DLB/PDD (N=97)

Risk Factor		Hallucinations [Mean ±SD / Frequency (%)]		Odd ratio (95% CI)	p-value
		No (n=51)	Yes (n=46)		
Age		76 (6)	76 (6.4)	1 (0.9-1.1)	0.7
Gender	Female	29 (56)	28 (61)	Reference	-
	Male	22 (44)	18 (39)	1.6 (0.7-3.5)	0.3
MMSE		20 (6.9)	19 (7)	0.97 (0.9-1.03)	0.97
5HTTLPR	SS	12 (24)	8 (17)	Reference	-
	LS	23 (45)	23 (50)	1.5 (0.5-4.4)	0.5
	LL	16 (31)	15 (33)	1.4 (0.5 -4.4)	0.6
5HTTLPR allele	S	47 (46)	39 (42)	Reference	-
	L	55 (54)	53 (58)	1.2 (0.66-2.05)	0.6

3.4.2.2 Multivariate analysis: 5HTTLPR

Multivariate analyses for delusions and hallucinations by 5HTTLPR genotype are shown in the two tables below. Although MMSE was not a significant predictor of hallucinations it was still included as a covariate due to evidence suggesting cognitive impairment is associated with psychotic symptoms in DLB/PDD. For delusions, the risk associated with the 5HTTLPR LL increased to 11.5 (Table 3-5), while for hallucinations there continued to be no effect of 5HTTLPR genotype (Table 3-6). Multivariate analysis by allele, controlling for MMSE, again showed a significant effect of the L allele with (OR: 3.27, df=1, 95% CI: 1.54-6.94, p=0.002).

Table 3-5 Logistic regression: 5HTTLPR persistent delusions (N=97)

Risk Factor		**Persistent delusions** [Frequency (%)]		**Odd ratio (95% CI)**	**p-value**
		No (n=68)	**Yes (n=29)**		
5HTTLPR	SS	18 (26)	2 (7)	Reference	-
	LS	31 (46)	15 (52)	5.3 (0.9-32)	0.07
	LL	19 (28)	12 (41)	11.5 (1.7-77)	0.01

Adjusted for MMSE

Table 3-6 Logistic regression 5HTTLPR persistent hallucinations (N=97)

Risk Factor		**Persistent hallucinations** [Frequency (%)]		**Odd ratio (95% CI)**	**p-value**
		No (n=51)	**Yes (n=46)**		
5HTTLPR	SS	12 (24)	8 (17)	Reference	-
	LS	23 (45)	23 (50)	1.4 (0.5-4.2)	0.5
	LL	16 (31)	15 (33)	1.5 (0.5-4.6)	0.5

Adjusted for MMSE

3.4.2.3 Role of 5HTTLPR polymorphism in DLB/PDD sub-phenotypes

To analyse whether 5HTTLPR was associated with a specific DLB/PDD with psychotic symptoms phenotype, genotype frequencies were compared between DLB/PDD individuals with persistent symptoms and cognitively normal controls and DLB/PDD without persistent symptoms and cognitively normal controls.

Genotype frequencies by the presence/absence of persistent symptoms and for cognitively normal controls are shown in Table 3-7. There was no overall difference in the genotype frequencies between patients with dementia and cognitively normal controls (χ^2=0.4, df=2, p=0.9).

Comparisons between DLB/PDD with psychosis and controls and DLB/PDD without persistent symptoms and controls did not show any significant differences (persistent delusions vs. control: χ^2=2, df=2, p=0.4; persistent hallucinations vs. control: χ^2=0.3, df=2, p=0.9; no delusions vs. control: χ^2=2.3, df=2, p=0.3; no hallucinations vs. control: χ^2=0.8, df=2, p=0.7).

Table 3-7 5HTTLPR genotype frequencies for DLB/PDD patients (by the presence/absence of symptoms) and controls (N=187)

		Persistent Hallucinations		**Persistent Delusions**		**Control**
		[Frequency (%)]				
		No (n=51)[1]	**Yes (n=46)[2]**	**No (n=68)[3]**	**Yes (n=29)[4]**	**(n=90)**
5HTTLPR	SS	12 (24)	8 (17)	18 (26)	2 (7)	16 (18)
	LS	23 (45)	23 (50)	31 (46)	15 (52)	41 (46)
	LL	16 (31)	15 (33)	19 (28)	12 (41)	33 (36)

1. no hallucinations vs. control: χ2=0.8, df=2, p=0.7; **2.** persistent hallucinations vs. control: χ^2=0.3, df=2, p=0.9; **3.** no delusions vs. control: χ^2=2.3, df=2, p=0.3; **4.** persistent delusions vs. control: χ^2=2, df=2, p=0.4

3.4.3 Primary analysis: *COMT* val158met

The analysis for the *COMT* val158met polymorphism followed the same plan as above for 5HTTLPR.

Twenty-nine individuals (31%) had persistent delusions and 68 (70%) were classified as having no delusions. Twenty-one (31%) of the no delusion group also had non-persistent delusions. MMSE among those with persistent delusions was significantly lower than those without (t = 4.3, p < 0.001). There were no differences in age (t = −1.3, p = 0.2) or gender (χ^2 = 0.4, df = 1, p = 0.6) in this group (Table 3-8, page 114).

Forty-six (47%) people had persistent hallucinations. Of the 51 (53%) people classified as having no hallucinations, 20 (39%) had non-persistent hallucinations. There were no differences in mean

age (t = −0.3, p = 0.8), mean MMSE (t = 0.8, p = 0.4) or gender (χ^2 = 1.1, df = 1, p = 0.3) between those with and without hallucinations (Table 3-9, page 115).

3.4.3.1 Univariate analysis: *COMT* val158met

In univariate analysis of persistent delusions, MMSE was the only significant predictor (Table 3-8).

Table 3-8 Logistic regression: persistent delusions in DLB/PDD (N=97)

Risk Factor		**Persistent delusions** [Mean ±SD / Frequency (%)]		**Odd ratio (95% CI)**	**p-value**
		No (n=70)	**Yes (n=27)**		
Age		78 (6.5)	76 (5.9)	1.1 (0.97-1.1)	0.2
Gender	Female	41 (59)	14 (52)	Reference	-
	Male	29 (41)	13 (48)	1.3 (0.5-3.2)	0.6
MMSE		21 (6.1)	15 (7.7)	0.9 (0.8-0.94)	<0.001
COMT	met/met	17 (24)	5 (19)	Reference	-
	val/met	41 (59)	16 (59)	1.9 (0.5-7.6)	0.3
	val/val	12 (17)	6 (22)	1.9 (0.6-5.8)	0.3
COMT allele	met	75 (54)	26 (48)	Reference	-
	val	65 (46)	28 (52)	2 (0.9-4.4)	0.1

In univariate analysis of persistent hallucinations, there were no significant predictors (Table 3-9).

Table 3-9 Logistic regression: persistent hallucinations in DLB/PDD (N=97)

Risk Factor		Hallucinations [Mean ±SD / Frequency (%)]		Odd ratio (95% CI)	p-value
		No (n=51)	Yes (n=46)		
Age		77 (5.7)	76 (6.4)	1.01 (0.9-1.1)	0.8
Gender	Female	31 (61)	23 (50)	Reference	-
	Male	23 (39)	23 (50)	1.6 (0.7-3.5)	0.3
MMSE		19 (7.1)	20 (7.1)	0.97 (0.9-1.03)	0.4
COMT	met/met	16 (31)	11 (24)	Reference	-
	val/met	23 (45)	27 (59)	0.9 (0.3-3.1)	0.9
	val/val	12 (23)	8 (17)	1.8 (0.7-4.6)	0.2
COMT allele	met	55 (54)	49 (53)	Reference	-
	val	47 (46)	43 (47)	1.7 (0.9-3.3)	0.1

3.4.3.2 Multivariate analysis: *COMT* val158met

In both multivariate logistic regression analyses MMSE was included as a covariate. There was still no significant effect of genotype on either persistent delusions or persistent hallucinations (Table 3-10 and Table 3-11 respectively).

Table 3-10 Logistic regression: *COMT* val158met persistent delusions (N=97)

Risk Factor		Persistent delusions [Frequency (%)]		Odd ratio (95% CI)	p-value
		No (n=68)	Yes (n=29)		
COMT	met/met	17 (24)	5 (19)	Reference	-
	val/met	41 (59)	16 (59)	1.2 (0.3-5.6)	0.8
	val/val	12 (17)	6 (22)	1.7 (0.5-5.7)	0.4

Adjusted for MMSE

Table 3-11 Logistic regression: *COMT* val158met persistent hallucinations (N=97)

Risk Factor		Persistent hallucinations [Frequency (%)]		Odd ratio (95% CI)	p-value
		No (n=51)	Yes (n=46)		
COMT	met/met	16 (31)	11 (24)	Reference	-
	val/met	23 (45)	27 (59)	0.86 (0.3-3)	0.8
	val/val	12 (23)	8 (17)	1.8 (0.7-4.6)	0.2

Adjusted for MMSE

3.4.3.3 Role of *COMT* val158met polymorphism in Lewy body dementia sub-phenotypes

To analyse whether *COMT* val158met was associated with a specific DLB/PDD with psychotic symptoms phenotype, genotype frequencies were compared between DLB/PDD individuals with persistent symptoms and cognitively normal controls and DLB/PDD without persistent symptoms and cognitively normal controls.

Genotype frequencies by the presence/absence of persistent symptoms and for cognitively normal controls are shown in Table 3-12. There was no overall difference in the genotype frequencies between cases with dementia and cognitively normal controls (χ^2=0.1, df=2, p=0.9).

Comparisons between DLB/PDD with psychosis and controls and DLB/PDD without persistent symptoms and controls did not show any significant differences (persistent delusions vs. control: χ^2=0.7, df=2, p=0.7; persistent hallucinations vs. control: χ^2=0.2, df=2, p=0.9; no delusions vs. control: χ^2=0.5, df=2, p=0.8; no hallucinations vs. control: χ^2=1.1, df=2, p=0.6).

Table 3-12 *COMT* val158met genotype frequencies for DLB/PDD cases (by the presence/absence of symptoms) and controls (N=189)

		Persistent Hallucinations		**Persistent Delusions**		**Control**
		[Frequency (%)]				
		No (n=51)[1]	**Yes (n=46)[2]**	**No (n=70)[3]**	**Yes (n=27)[4]**	**(n=92)**
COMT	met/met	14 (31)	12 (24)	21 (30)	5 (19	24 (26)
	val/met	21 (45)	30 (59)	34 (49)	16 (59)	50 (54)
	val/val	11 (24)	9 (17)	15 (21)	6 (22)	18 (20)

1. no hallucinations vs. control: $\chi 2=1.1$, df=2, p=0.6; **2.** persistent hallucinations vs. control: $\chi 2=0.2$, df=2, p=0.9; **3.** no delusions vs. control: $\chi 2=0.5$, df=2, p=0.8; **4.** persistent delusions vs. control: $\chi 2=0.7$, df=2, p=0.7

3.4.4 Secondary analysis

The secondary analysis consisted of a sensitivity analysis of the results presented in sections 3.4.2 and 3.4.3. The split of symptoms used in the primary analysis was evaluated by only testing those with persistent symptoms and those with no symptoms (i.e. transient symptom cases were excluded). For the *COMT* polymorphism, this made no difference to the results, they were still not significant for both hallucinations (val/met: OR 0.8, 95% CI 0.2-3.4, p=0.8; val/val: OR 1.2, 95% CI 0.4-3.6, p=0.4) and delusions (val/met: OR 0.8, 95% CI 0.2-4.3, p=0.8; val/val: OR 1.4, 95% CI 0.4-5.4, p=0.6).

For 5HTTLPR, the LL genotype still carried an increased risk for persistent delusions (OR: 14, 95% CI: 1.5-131, p=0.02), but not persistent hallucinations where ORs were similar to those presented in section 3.4.2.2 (LS: OR 1.6, 95% CI 0.5-5.6, p=0.4; LL: OR 1.7, 95% CI 0.5-6.3, p=0.4). Although it should be acknowledged that this analysis reduced the sample size to 78, hence the very wide confidence intervals.

3.4.5 Summary

3.4.5.1 Summary of primary analysis

1. LL genotype and L allele were associated with an 11.5- and 2-fold increased risk respectively for persistent delusions in DLB/PDD. There was no association with hallucinations.
2. There was no evidence to support the hypothesis that either polymorphism is associated with an increased risk for a DLB/PDD with psychotic symptoms phenotype.

3.4.5.2 Summary of secondary analysis

1. Restricting the analysis to only those with persistent symptoms and those with no symptoms did not change the results significantly: The association with the LL genotype persisted and there was still no association with hallucinations and the 5HTTLPR polymorphism or any symptom and the *COMT* val158met polymorphism.

3.5 Results: Alzheimer's disease

3.5.1 Descriptive statistics

Of the 1,353 cases from the nine databases eligible for screening, 584 met the inclusion/exclusion criteria for this study. Of these, 10 patients were known to be of an ethnic origin other than European and were excluded, leaving 574 AD patients in the final analysis. One-hundred and fifteen were of unknown ethnic origin but were retained for the analysis given the extremely low frequency of non-Europeans among those with known ethnicities (2%). A pathological diagnosis of AD was available for 95 patients.

One hundred and eleven cognitively normal controls were available from the ANM and POCD cohorts. They did not differ significantly by gender frequency (60% female: χ^2=1.4, df=1, p=0.2) or age (mean 78: t=-1.3, df=683, p=0.2).

Genotype frequencies for both *COMT* val158met and 5HTTLPR did not deviate from Hardy-Weinberg equilibrium (5HTTLPR: χ^2=1.7, p>0.05; COMT: χ^2=0.4, p>0.05).

Participant demographics by *COMT* val158met and 5HTTLPR genotype are present in Table 3-13 and
Table 3-14 respectively. There were no significant differences in frequencies of means of any of the variables presented for *COMT* val158met (age: *F(*2,571)=1.7, p=0.2; gender: χ^2=0.5, df=2, p=0.8; dementia severity: χ^2=7.5, df=4, p=0.1; anti dementia drug: χ^2=3.8, df=2, p=0.1; antipsychotic: χ^2=0.3, df=3, p=0.9) or 5HTTLPR (age: *F(*2,571)=0.6, p=0.6; gender: χ^2=3.1, df=2, p=0.2; dementia severity: χ^2=2.6, df=4, p=0.6; anti dementia drug: χ^2=1, df=2, p=0.6; antipsychotic: χ^2=0.9, df=3, p=0.6).

Table 3-13 Participant demographics by *COMT* genotype (N=574)

		***COMT* val158met** [Mean±SD / Frequency(%)]		
		val/val (n=143)	**val/met (n=280)**	**met/met (n=151)**
Age[1]		78 (8.8)	80 (8)	79 (8.2)
Gender[2]	Female	95 (66)	187 (67)	96 (64)
	Male	48 (34)	93 (33)	55 (36)
Dementia severity[3]	Mild	38 (27)	74 (26)	51 (34)
	Moderate	38 (27)	76 (27)	49 (32)
	Severe	67 (46)	130 (46)	51 (34)
Anti dementia drug[4]	No	103 (72)	178 (64)	106 (70)
	Yes	40 (28)	102 (36)	45 (30)
Antipsychotic[5]	No	82 (57)	168 (60)	88 (58)
	Yes	61 (43)	112 (40)	63 (42)

1. $F(2,571)=1.7$, p=0.2; **2.** $\chi^2=0.5$, df=2, p=0.8; **3.** $\chi^2=7.5$, df=4, p=0.1; **4.** $\chi^2=3.8$, df=2, p=0.1; **5.** $\chi^2=0.3$, df=3, p=0.9

Table 3-14 Participant demographics by 5HTTLPR genotype (N=574)

		5HTTLPR [Mean±SD / Frequency(%)]		
		SS (n=120)	**LS (n=273)**	**LL (n=181)**
Age[1]		79 (8.2)	80 (8.6)	79 (7.8)
Gender[2]	Female	86 (72)	171 (63)	121 (67)
	Male	34 (28)	102 (37)	60 (33)
Dementia severity[3]	Mild	38 (32)	73 (27)	52 (29)
	Moderate	37 (31)	80 (29)	46 (25)
	Severe	45 (38)	120 (44)	83 (46)
Anti dementia drug[4]	No	77 (64)	189 (69)	121 (67)
	Yes	43 (36)	84 (31)	60 (33)
Antipsychotic[5]	No	66 (55)	163 (60)	109 (60)
	Yes	54 (45)	110 (40)	72 (40)

1. $F(2,571)=0.6$, p=0.6; **2.** $\chi^2=3.1$, df=2, p=0.2; **3.** $\chi^2=2.6$, df=4, p=0.6; **4.** $\chi^2=1$, df=2, p=0.6
5. $\chi^2=0.9$, df=3, p=0.6

Delusion ratings were available for the full 574 patients while one patient was missing hallucination ratings, meaning 573 cases were included in the analysis of hallucinations.

Seventy-eight (14%) and 119 (21%) cases were classified as having persistent hallucinations and delusions respectively. One-hundred and nine (19%) and 156 (27%) were classified as having transient hallucinations and delusions respectively.

For the primary analysis the transient symptom group and no symptom group were analysed together as a no symptom group. Among those without persistent delusions 37 (8%) had persistent hallucinations, while there were twice as many cases with persistent delusions among those without persistent hallucinations (78, 16%).

3.5.2 Primary analysis

3.5.2.1 Univariate analysis

One hundred and nineteen (21%) individuals had persistent delusions and of the 299 (52%) classified has having no delusions 156 (27%) had transient delusions. Patients with transient delusions were grouped with the no delusion group. Forty-one (34%) of those with persistent delusions and 37 (8%) of those with no delusions also had persistent hallucinations.

The univariate analyses for persistent delusions and hallucinations are presented in Table 3-15. Taking an anti-dementia drug was associated with a 2-fold increased risk of persistent delusions. No other risk factors were associated with persistent delusions, including 5HTTLPR and *COMT* genotypes or alleles.

Table 3-15 Logistic regression: persistent delusions in AD (N=574)

Risk Factor		Persistent delusions [Mean ±SD / Frequency (%)]		Odd ratio (95% CI)	p-value
		No (n=455)	Yes (n=119)		
Age		79 (8.2)	78 (8.4)	0.97 (0.9-1)	0.08
Gender	Female	298 (65)	80 (67)	Reference	-
	Male	157 (35)	39 (33)	0.9 (0.6-1.4)	0.7
Dementia severity	Mild	137 (30)	26 (22)	Reference	-
	Moderate	125 (27)	38 (32)	1.6 (0.9-2.8)	0.1
	Severe	193 (42)	55 (46)	1.5 (0.9-2.5)	0.1
Antipsychotic	No	267 (59)	71 (60)	Reference	-
	Yes	188 (41)	48 (40)	0.96 (0.6-1.5)	0.8
Anti dementia drug	No	322 (71)	65 (55)	Reference	-
	Yes	133 (29)	54 (45)	2 (1.3-3)	0.001
COMT	met/met	118 (26)	33 (28)	Reference	-
	val/met	222 (49)	58 (49)	0.9 (0.6-1.5)	0.8
	val/val	115 (25)	28 (24)	0.9 (0.5-1.5)	0.6
5HTTLPR	SS	94 (21)	26 (21)	Reference	-
	LS	214 (47)	59 (50)	0.9 (0.6-1.7)	0.9
	LL	147 (32)	34 (29)	0.8 (0.5-1.5)	0.5
COMT allele	A	458 (50)	124 (52)	Reference	-
	G	452 (50)	114 (48)	0.9 (0.7-1.2)	0.6
5HTTLPR allele	S	402 (44)	111 (47)	Reference	-
	L	508 (56)	127 (53)	0.9 (0.7-1.2)	0.5

Seventy-eight individuals (14%) were classified as having persistent hallucinations. Of the 386 (67%) people classified as having no hallucinations, 109 (22%) had transient hallucinations. Among those with persistent hallucinations 41 (52%) also had persistent delusions, while in the no hallucinations group 78 (16%) had persistent delusions.

With regard to persistent hallucinations, both younger age and severe dementia stage carried a significantly increased risk (OR: 0.96, 95% CI: 093-0.98, p=0.004; OR: 3.3, 95% CI: 1.7-6.6, p=0.001). Again, no other risk factors were associated with persistent hallucinations (Table 3-16).

Table 3-16 Logistic regression: persistent hallucinations in AD (N=573)

Risk Factor		Hallucinations [Mean ±SD / Frequency (%)]		Odd ratio (95% CI)	p-value
		No (n=495)	Yes (n=78)		
Age		80 (8.1)	77 (8.9)	0.96 (0.93-0.98)	0.004
Gender	Female	324 (65)	53 (68)	Reference	
	Male	171 (35)	25 (32)	0.9 (0.5-1.5)	0.7
Dementia severity	Mild	152 (31)	11 (14)	Reference	-
	Moderate	144 (29)	19 (24)	1.8 (0.8-4)	0.1
	Severe	199 (40)	48 (62)	3.3 (1.7-6.6)	0.001
Antipsychotic	No	297 (60)	40 (51)	Reference	-
	Yes	198 (40)	38 (49)	1.4 (0.9-2.3)	0.1
Anti dementia drug	No	339 (68)	47 (60)	Reference	
	Yes	156 (32)	31 (40)	1.4 (0.9-2.3)	0.2
COMT	met/met	131 (26)	20 (26)	Reference	-
	val/met	243 (49)	36 (46)	0.97 (0.54-1.7)	0.9
	val/val	121 (24)	22 (28)	1.2 (0.6-2.3)	0.6
5HTTLPR	SS	104 (21)	16 (21)	Reference	-
	LS	231 (47)	41 (53)	1.2 (0.6-2.1)	0.7
	LL	160 (32)	21 (27)	0.9 (0.4-1.7)	0.7
COMT allele	met	505 (51)	76 (49)	Reference	-
	val	485 (49)	80 (51)	1.1 (0.8-1.5)	0.6
5HTTLPR allele	S	439 (44)	73 (47)	Reference	-
	L	551 (56)	83 (53)	0.9 (0.7-1.2)	0.6

3.5.2.2 Multivariate analysis

Although dementia severity was not associated with an increased risk for delusions it was still included as a covariate because of the strong association highlighted in previous research. Along with dementia severity, anti-dementia drug exposure highlighted from the univariate analysis was also included as a covariate. For the hallucination analysis, age and dementia severity were included as covariates.

Multivariate analyses for delusions and hallucinations by 5HTTLPR and *COMT* genotype are shown in the four tables below (Table 3-17 to Table 3-20). There continued to be no evidence of an association with either genotype and either symptom.

Table 3-17 Logistic regression: *COMT* val158met persistent delusions (N=574)

Risk Factor		**Persistent delusions** [Frequency (%)]		**Odd ratio (95% CI)**	**p-value**
		No (n=455)	**Yes (n=119)**		
COMT	met/met	118 (26)	33 (28)	Reference	-
	val/met	222 (49)	58 (49)	0.9 (0.5-1.4)	0.5
	val/val	115 (25)	28 (24)	0.8 (0.5-1.5)	0.5

Adjusted for dementia severity and anti-dementia drug exposure

Table 3-18 Logistic regression: 5HTTLPR persistent delusions (N=574)

Risk Factor		**Persistent delusions** [Frequency (%)]		**Odd ratio (95% CI)**	**p-value**
		No (n=455)	**Yes (n=119)**		
5HTTLPR	SS	94 (21)	26 (21)	Reference	-
	LS	214 (47)	59 (50)	1.02 (0.6-1.7)	0.9
	LL	147 (32)	34 (29)	0.8 (0.5-1.5)	0.5

Adjusted for dementia severity and anti-dementia drug exposure

Table 3-19 Logistic regression: COMT persistent hallucinations (N=573)

Risk Factor		Persistent hallucinations [Frequency (%)] No (n=495)	Yes (n=78)	Odd ratio (95% CI)	p-value
COMT	met/met	243 (49)	36 (46)	Reference	-
	val/met	121 (24)	22 (28)	0.9 (0.5-1.6)	0.6
	val/val	104 (21)	16 (21)	1 (0.5-1.9)	0.9

Adjusted for dementia severity and age

Table 3-20 Logistic regression 5HTTLPR persistent hallucinations (N=573)

Risk Factor		Persistent hallucinations [Frequency (%)] No (n=495)	Yes (n=78)	Odd ratio (95% CI)	p-value
5HTTLPR	SS	104 (21)	16 (21)	Reference	-
	LS	231 (47)	41 (53)	1.1 (0.6-2.1)	0.8
	LL	160 (32)	21 (27)	0.78 (0.4-1.6)	0.5

Adjusted for dementia severity and age

3.5.2.3 Role of *COMT* val158met and 5HTTLPR polymorphisms in AD sub-phenotype

To analyse whether each of the polymorphisms was associated with a specific AD with psychotic symptoms phenotype, genotype frequencies were compared between AD individuals with persistent symptoms and cognitive normal controls and AD without persistent symptoms and cognitively normal controls.

Genotype frequencies by the presence/absence of psychotic symptoms and controls for 5HTTLPR and *COMT* val158met are shown in Table 3-21 and Table 3-22 respectively. There was no overall difference in frequencies between AD cases and controls for either 5HTTLPR or *COMT* val158met (χ^2=0.4, df=2, p=0.8 and χ^2=0.7, df=2, p=0.7 respectively).

There was no evidence to support the heterogeneity model for either 5HTTLPR (persistent delusions vs. control: χ^2=0.5, df=2, p=0.8; persistent hallucinations vs. control: χ^2=1.05, df=2, p=0.6; no delusions vs. control: χ^2=0.7, df=2, p=0.7; no hallucinations vs. control: χ^2=0.6, df=2, p=0.7) or *COMT* val158met (persistent delusions vs. control: χ^2=0.05, df=2, p=0.9; persistent

hallucinations vs. control: χ^2=0.8, df=2, p=0.6; no delusions vs. control: χ^2=0.6, df=2, p=0.8; no hallucinations vs. control: χ^2=0.3, df=2, p=0.8).

Table 3-21 5HTTLPR genotype frequencies for AD cases (by presence/absence of symptoms) and controls (N=685)

		Persistent Hallucinations		**Persistent Delusions**		**Control**
		[Frequency (%)]				
		No (n=495)[1]	**Yes (n=78)[2]**	**No (n=455)[3]**	**Yes(n=119)[4]**	**(n=111)**
5HTTLPR	SS	104 (21)	16 (20)	94 (21)	26 (22)	34 (31)
	LS	231 (47)	41 (53)	214 (47)	59 (50)	50 (45)
	LL	160 (32)	21 (27)	147 (32)	34 (28)	27 (24)

1. no hallucinations vs. control: χ2=0.6, df=2, p=0.7; **2.** persistent hallucinations vs. control: χ2=1.05, df=2, p=0.6; **3.** no delusions vs. control: χ2=0.7, df=2, p=0.7; **4.** persistent delusions vs. control: χ2=0.5, df=2, p=0.8

Table 3-22 *COMT* val158met genotype frequencies for AD cases (by presence/absence of symptoms) and controls (N=685)

		Persistent Hallucinations		**Persistent Delusions**		**Control**
		[Frequency (%)]				
		No (n=495)[1]	**Yes (n=78)[2]**	**No (n=455)[3]**	**Yes(n=119)[4]**	**(n=111)**
COMT	Met/met	131 (26)	20 (26)	118 (26)	33 (28)	32 (29)
	Val/met	243 (49)	36 (46)	222 (49)	58 (49)	54 (49)
	Val/val	121 (24)	22 (28)	115 (25)	28 (23)	25 (22)

1. no hallucinations vs. control: χ2=0.3, df=2, p=0.8; **2.** persistent hallucinations vs. control: χ2=0.8, df=2, p=0.6; **3.** no delusions vs. control: χ2=0.6, df=2, p=0.8; **4.** delusions vs. control: χ2=0.05, df=2, p=0.9

3.5.3 Secondary analysis

The secondary analysis consisted of a sensitivity analysis of the results presented in section 3.5.2. The split of symptoms used in the primary analysis was evaluated by only testing those with persistent symptoms and those with no symptoms (i.e. transient symptom cases were excluded). This made no difference to the results, the ORs and p-values were similar to those presented in section 3.5.2.2 for both delusions (*COMT* val/met: OR 0.9, 95% CI: 0.6-1.6, p=0.8; *COMT* val/val: OR 0.8, 95% CI: 0.4-1.5, p=0.5; 5HTTLPR LS: OR 1.2, 95% CI: 0.7-2, p=0.5; 5HTTLPR LL: OR 1.2, 95% CI: 0.6-2.1, p=0.7) and hallucinations (*COMT* val/met: OR 0.8, 95%

CI: 0.4-1.6, p=0.6; *COMT* val/val: OR 0.97, 95% CI: 0.5-2, p=0.9; 5HTTLPR LS: OR 1.5, 95% CI: 0.8-2.6, p=0.2; 5HTTLPR LL: OR 1.1, 95% CI: 0.5-2.4, p=0.7).

All of the OPTIMA patients were evaluated using the CAMDEX scale, while the remainder were assessed using the NPI. Thus in the second part of the secondary analysis these cases were excluded in order to test for any bias arising from measurement of symptoms on different rating scales. Again this made no significant differences to the results for delusions (*COMT* val/met: OR 0.8, 95% CI: 0.4-1.3, p=0.3; *COMT* val/val: OR 0.8, 95% CI: 0.4-1.5, p=0.5; 5HTTLPR LS: OR 1.2, 95% CI: 0.7-2, p=0.6; 5HTTLPR LL: OR 1.3, 95% CI: 0.7-2.4, p=0.4) or hallucinations (*COMT* val/met: OR 0.8, 95% CI: 0.4-1.6, p=0.6; *COMT* val/val: OR 0.7, 95% CI: 0.3-1.6, p=0.4; 5HTTLPR LS: OR 1.3, 95% CI: 0.7-2.7, p=0.4; 5HTTLPR LL: OR 1.5, 95% CI: 0.7-3.4, p=0.3).

3.5.4 Summary

3.5.4.1 Summary of primary analysis

1. No association was found between either the 5HTTLPR or *COMT* val158met polymorphisms and persistent delusions or hallucinations in AD.

3.5.4.2 Summary of secondary analysis

1. Restricting the analysis to only those with persistent symptoms and no symptoms or to only those cases which were assessed using the NPI made no difference to the result.

3.6 Discussion

This is the first study to show that the LL genotype of the 5HTTLPR polymorphism is a risk factor for persistent delusions in DLB/PDD patients, while there was no association between the *COMT* val158met polymorphism and either symptom. Individuals carrying the LL genotype were 11.5 times more likely to have persistent delusions than individuals homozygous for the S allele and for each one point decrease in MMSE score persistent delusions risk increased significantly by a factor of 1.1. Furthermore, sensitivity analysis demonstrated that the L allele was associated with a 3.3 fold increase in risk for persistent delusions relative to the S allele. In contrast, neither 5HTTLPR genotype nor MMSE score were significant predictors of persistent hallucinations, demonstrating that these symptoms may have distinct underlying mechanisms, a finding supported by post-mortem and in-vivo imaging studies (Nagahama, Okina et al. 2010, Ballard, Piggott et al. 2000). Moreover, there was no association found between the *COMT* val158met polymorphism and delusions or hallucinations in DLB/PDD.

In contrast there was no evidence to support a role for the 5HTTLPR polymorphism in the aetiology of persistent delusions and hallucinations in AD. This was both in univariate and multivariate analyses after controlling for dementia severity. This is in contrast to a substantial body of previous literature (Sweet, Pollock et al. 2001, Borroni, Grassi et al. 2006, Quaranta, Bizzarro et al. 2009, Proitsi, Lupton et al. 2010), although it should be noted that there have also been some negative reports previously (Albani, Prato et al. 2009, Rocchi, Micheli et al. 2003, Ueki, Ueno et al. 2007). The fact that the positive previous findings are in opposite directions may reflect the complexity of the psychotic phenotype, an issue which has been addressed in this study by the use of serial assessment and the adoption of stringent criteria for the classification of a symptom as present. Therefore this finding can be considered a robust negative.

As expected neither *COMT* val158met nor 5HTTLPR were associated with AD or DLB/PDD. Moreover, there was no evidence to suggest that either of these polymorphisms represent an increased risk for a dementia with psychotic symptoms sub-phenotype (the heterogeneity model). This analysis of course rests on the assumption that such a phenotype does exist and although not been conclusively proven there is evidence to support this (Sweet, Nimgaonkar et al. 2003). Moreover the control group comparison in this study was from the UK while the dementia groups

were from the UK and other parts of Europe, this is a significant limitation as it makes population stratification more likely and therefore the results of this secondary, minor analysis should be treated with caution. Therefore, it appears that both polymorphisms have no involvement in any kind of delusion or hallucination phenotype in AD. In DLB/PDD the evidence presented here suggests that, similarly, *COMT* val158met does not contribute to any hallucination or delusion phenotype while the role of 5HTTLPR appears to be restricted to that of disease modification; causing an increased risk for persistent delusions, but not hallucinations.

Review of the previous literature strongly suggests that both *COMT* val158met and 5HTTLPR are pleiotropic, influencing a number of traits and disorders besides psychosis. Therefore, for a complete understanding of the role of these polymorphisms in psychosis in dementia their role in traits that are associated with psychotic symptoms should be investigated. Of particular note is the body of literature surrounding both *COMT* val158met and 5HTTLPR, and more broadly serotoninergic and dopaminergic neurotransmission, and cognitive decline in AD. Indirect associations with psychosis such as this cannot be ruled out and indeed may be one reason for the inconsistency in the literature surrounding 5HTTLPR and *COMT* val158met, however, below the specific reasons for the findings reported here will be discussed.

3.6.1 *COMT* val158met

There have been two positive reports of the *COMT* val allele or val/val genotype being associated with psychosis in AD (Borroni, Grassi et al. 2006, Sweet, Devlin et al. 2005, Proitsi, Lupton et al. 2010). In DLB and PDD this area is less well studied. The only previous genetic association study in DLB reported a significant association between *COMT* val158met and psychosis (delusions or hallucinations), but a further evaluation of delusions and hallucinations separately did not show a significant relationship (Borroni, Di Luca et al. 2006). Camicioli et al. (Camicioli, Rajput et al. 2005) also failed to find an association with *COMT* val158met in PD patients with visual hallucinations.

From a neurochemical point of view, the negative *COMT* val158met finding in both AD and DLB/PDD is perhaps not surprising. There is little evidence that dopaminergic drugs are effective in the treatment of psychosis in AD or DLB/PDD, with primarily non-dopaminergic drugs offering

the best response profile in both diseases (Corbett, Smith et al. 2012, Ballard, Aarsland et al. 2013). The best evidence in AD is for atypical antipsychotics which are characterised by high affinity $5HT_{2A}$ receptor antagonism, rather than solely by D2 antagonism as is the case for typical antipsychotic agents, and in DLB/PDD cholinesterase inhibitors appear to confer the greatest benefit. While the exact mechanism of benefit of atypical antipsychotic agents is unclear because of their actions at multiple receptor sites, this DA activity-based distinction between typical and atypicals may be of relevance, suggesting a relative importance of 5HT modulation in antipsychotic action. Furthermore, DLB/PDD levodopa studies showing no evidence of increased psychosis prevalence following administration of this drug support the notion that CNS dopamine levels do not underpin psychosis (Goetz, Pappert et al. 1998). To explore this further future studies should examine the relationship between dopamine and cholinergic receptor polymorphisms and psychosis, in particular in *DRD1*, *DRD3* and *CHRNA7* as these have been associated with psychosis in AD (Sweet, Nimgaonkar et al. 1998, Holmes, Smith et al. 2001, Carson, Craig et al. 2008).

3.6.2 5HTTLPR

Preliminary evidence in PD patients with psychosis in the absence of dementia suggests that the $5HT_{2A}$ inverse agonist pimavanserin may confer benefits in the treatment of psychotic symptoms, with a preferential response to delusions (Meltzer, Mills et al. 2010, Cummings, Isaacson et al. 2013). Pimavanserin is a very selective $5HT_{2A}$ blocker with no dopaminergic activity and its efficacy is therefore supportive of a key role of serotonergic neurotransmission at $5HT_{2A}$ in the aetiology of psychosis in PD. Whether it is also effective in DLB/PDD is not yet known, but that 5HTTLPR was associated with delusions in this study highlights the possibility of extending this line of research, and indicates the potential value of treatments targeting the 5HT system for the treatment of persistent delusions in DLB and PDD patients (Meltzer, Mills et al. 2010). The evidence from the pimavanserin trials is supportive of the excess $5HT_{2A}$ receptor stimulation hypothesis of psychosis but it is not clear how the 5HTTLPR polymorphism may fit into this mechanism. If the L allele is associated with greater efficiency in removal of 5HT from the synaptic cleft, thus decreasing serotonergic neurotransmission relative to the S allele, then one would expect it to be protective against psychosis as this action is more in line with pimavanserin as a $5HT_{2A}$ antagonist. Serotonergic neurotransmission is complex and not completely

understood with respect to the aetiology of psychosis, however there are other plausible attributes of 5HTTLPR that may serve to explain this finding in the context of the excess serotonin hypothesis. There is evidence from two PET imaging studies that the 5HTTLPR S allele is associated with a higher density of $5HT_{1A}$ receptors in some areas of the neocortex (including frontal and temporal areas) and the cingulate (David, Murthy et al. 2005, Lee, Bailer et al. 2005, Lothe, Boni et al. 2009). A mechanistic explanation of psychosis involving $5HT_{1A}$ is supported by evidence that increased $5HT_{1A}$ activation in the frontal cortex resulting from $5HT_{2A}$ and D2 blockade is a key property of atypical antipsychotics (Ichikawa, Ishii et al. 2001). Accordingly, lower numbers of $5HT_{1A}$ receptors and lower concentrations of 5HT associated with the L allele may lead to less signalling at $5HT_{1A}$ thereby conferring less resistance to psychosis among carriers of this allele.

A phase II trial of pimavanserin for psychotic symptoms in AD is to commence within the next year and based on its pharmacological profile and efficacy in PD positive results are expected. Along with the other evidence implicating serotonergic neurotransmission in psychotic symptoms in AD, it is therefore somewhat surprising that no association was found in this study between 5HTTLPR and psychotic symptoms in AD. This is difficult to interpret but given the design of this study this should be considered a robust negative and in light of this the possibility that the positive finding in the DLB/PDD cohort was a false positive should not be ignored. Nonetheless, there are several strengths which should also be acknowledged, these will be discussed in more detail below.

The results relating to AD do not rule out serotonergic dysfunction in the aetiology of psychotic symptoms, they do however strongly suggest that 5HTTLPR is not involved. Polymorphisms in the $5HT_{2A}$ gene were not examined in this study but present an interesting avenue for future research. The common $5HT_{2A}$ T102C SNP is relatively well studied in AD psychosis, it is a synonymous SNP and although there is a promoter variant which has been shown to be in complete linkage disequilibrium (Spurlock, Heils et al. 1998), because of the known biological effects of 5HTTLPR and the equal amount of previous literature as $5HT_{2A}$ the decision was taken to investigate it here. Given the recent evidence surrounding pimavanserin and the plans to commence trials in AD it would be of great interest to examine the role of this polymorphism and indeed the $5HT_{2A}$ gene in psychosis in AD.

3.6.3 Strengths and limitations

The strengths of this design include the prospective follow up of individuals to ensure accurate clinical data, assessments of cognitive impairment and controlling for it in the statistical analysis. This is the largest DLB/PDD genetic association study focussing on neuropsychiatric symptoms to date and among the largest in AD although the number of patients was modest compared with many genetic association studies in other areas. The number of individuals eligible was restricted by the stringent criteria for persistent psychotic symptoms which were adopted to increase power by ensuring accurate symptom classification, an issue which has presented substantial problems in retrospective studies. This is particularly evident in the AD cohort where over 1300 patients were screened but only 574 met the criteria. However it was felt necessary to adopt these criteria in order to increase the confidence with which cases were assigned to groups.

A further strength of this study is that all groups were matched on the demographic variables. There were however differences with respect to cognitive function, where those with delusions had a significantly lower MMSE than those without in the DLB/PDD cohort. The same bias was also apparent for hallucinations in the AD cohort (i.e. in those with more severe dementia there was a significantly higher proportion of people with hallucinations), see sections 3.4.2, 3.4.3 and 3.5.2. The inclusion of MMSE (DLB/PDD) or dementia severity (AD) as an explanatory variable statistically mitigated its effect on the genotypic association, however there is a risk that symptoms in some individuals with mild dementia scores may not have had sufficient time to emerge. For this reason a post-hoc analysis was undertaken where patients with an MMSE >19 or with mild dementia measured by FAST who had not already presented with delusions or hallucinations were excluded. This still supported the presence of a significant association with the LL genotype and delusions in the DLB/PDD cohort (n=47, χ^2=7.15, df=2, p=0.03), however it made no difference to any of the other comparisons in DLB/PDD or AD.

Comprehensive standardised drug histories for the participants in the DLB/PDD cohorts that came from brain banks were not available. Although there is limited evidence suggesting levodopa induces psychosis, it is possible that other drugs used for the treatment of PD could

contribute to psychosis and this may have impacted these results. Issues such as this should be addressed in future cohorts with detailed medication histories attached. In contrast, cases in the AD cohort did include details of medical history which allowed the identification of anti-dementia drug exposure as a predictor of persistent delusions (Table 3-15), which could then be controlled for in the multivariate analysis.

It is not uncommon for genetic association studies not to be replicated and there can be a number of reasons for this, the most obvious of which is ethnicity, which applied to both the DLB/PDD and AD parts of the study. This a broad issue and will be returned to in detail in the General Discussion. Another reason for non-replication is phenotype classification. There are a number of different ways in which psychotic symptoms, or indeed any neuropsychiatric symptoms, can be classified for analysis. With regard to 5HTTLPR and *COMT* val158met Sweet et al. (2001 and 2005), Quaranta et al. (2009) and Borroni et al. (2006) all used a psychosis phenotype which consisted of delusions and/or hallucinations. Proitsi et al. (2010) considered psychosis, delusions and hallucinations separately and instead of treating psychosis as a binary variable they treated it as continuous level data. With respect to 5HTTLPR, of the studies reporting significant associations, two identified the L allele/LL genotype as the risk (Sweet, Pollock et al. 2001, Quaranta, Bizzarro et al. 2009) and one the S allele/SS genotype (Borroni, Grassi et al. 2006). With respect to *COMT* val158met, both Sweet et al. (2005) and Borroni et al. (2006) found the val allele to carry the increased risk. Proitsi et al. (2010) found no association with either polymorphism and delusions or hallucinations but did report a significant *COMT* met/met*5HTTLPR SS genotype interaction on psychosis. Although in contrast to most of the previous literature the present study examined delusions and hallucinations separately and the classification of symptoms was based on stringent criteria designed to increase sensitivity and specificity which lend more confidence to the finding of no association with *COMT* val158met or 5HTTLPR.

Patients were only selected for inclusion in these studies if they had at least two assessments covering a period of at least six months. This criterion is strict and applied not only to those with psychotic symptoms but also to those without. Psychotic symptoms in AD are persistent, being likely to recur once they have emerged (Ballard, O'Brien et al. 2001, Craig, Mirakhur et al. 2005). Estimates of familial aggregation and heritability also provide support for this method being

applied. Specifically, estimates of both are highest for multiple or recurrent symptoms, suggesting these may have the strongest genetic basis (Sweet, Bennett et al. 2010, Bacanu, Devlin et al. 2005, Sweet, Nimgaonkar et al. 2002). Conversely, psychotic symptoms occurring transiently and not recurring may be more likely to be part of a concurrent delirium.

Multiple symptoms (i.e. a psychosis syndrome) were not examined in this study although there were a number of individuals who experienced both symptoms (see section 3.4.2 for DLB/PDD and 3.5.2 for AD). It could be argued that the familial and heritability evidence supports the concept of a psychotic syndrome that should be examined in genetic studies. However there is also neuropathological and neurochemical evidence that separate mechanisms underpin different psychotic symptoms in AD and DLB/PDD (Ballard, Piggott et al. 2000, Reeves, Brown et al. 2009, Christie, Shofer et al. 2012) and it is important that genetic association studies are used to explore this, which was one of the aims of the present study. Moreover, recent evidence from genetic association studies in AD supports treating delusions and hallucinations separately. Christie et al. (2012) reported an association between the *APOE*4* allele and patients with hallucinations (most of whom also had delusions) but not with delusions only.

Several different rating scales were used as a basis for symptom classification in the present study and as a result a binary present/absent coding symptom was employed. Again because of the different scales used, the broadest criterion was employed to determine whether a symptom was present or absent; that is the severity of any symptom was not taken into account. This is a potential limitation as it is possible that psychotic symptoms are a quantitative trait, however even if this were the case the ratings scales used in each of these studies may not be appropriate. The CAMDEX and CUSPAD scales only rate the presence of a symptom, while the PBE rates the frequency of symptoms over the past 28 days; all are ordinal or categorical level scales. The NPI composite score (the product of the frequency and severity scores) is often treated as a continuous variable (Proitsi, Lupton et al. 2012, Assal, Alarcon et al. 2004). However there are only nine possible scores: 0, 1, 2, 3, 4, 6, 8, 9, 12 and the difference between a score of 8 and 12 is not necessarily the same as the difference between 2 and 6. By using existing cohorts the design of the study presented here was necessarily limited by the data collected in each composite study. Therefore by using binary ratings any issues resulting from the treatment of the NPI as an interval level measurement were avoided and in order to capture the persistence of

symptoms, which appears to constitute a more heritable phenotype, the six month criterion was adopted.

3.6.4 Conclusion

With regard to DLB/PDD, these findings demonstrate that the 5HTTLPR polymorphism is associated with persistent delusions, which provides important mechanistic information to inform the development of new treatment approaches and the prioritisation and design of clinical trials.

In AD, although negative, this finding is robust due to the comprehensive clinical assessments from which the symptom phenotypes were drawn and therefore strongly suggests that 5HTTLPR and *COMT*val158met are not associated with delusions or hallucinations. It is also strengthened by a secondary analysis restricted to only those with no symptom and those with persistent symptom which showed no difference. Other genetic variation may be exerting influences in the different populations studied; again this will be returned to in the General Discussion. Moreover, at the phenotype level the next important stage in this line of research is to examine whether each of these polymorphisms is associated with other clinical correlates of psychotic symptoms, notably cognitive decline, in order to establish whether there is an indirect association present. This will be evaluated in the following chapter.

Chapter 4 *COMT* val158met is associated with rate of cognitive decline in Alzheimer's disease

4.1 Summary introduction

It has been proposed that AD with psychosis represents a distinct form of the disease (Sweet, Nimgaonkar et al. 2003). One of the key tenets of this hypothesis is the link between cognitive impairment and rate of cognitive decline and psychosis, particularly as the former appears to also precede the onset of the latter (Paulsen, Salmon et al. 2000).

Dopamine and serotonin are both key neurotransmitters which have been implicated in the pathogenesis of psychosis in AD. Two polymorphisms that may regulate neurotransmission in each of these systems have also been associated with psychotic symptoms (*COMT* val158met and 5HTTLPR). However, both serotonin and dopamine are also implicated in cognitive functioning. It therefore follows that if psychotic symptoms in AD are actually part of a phenotype that includes more rapid cognitive decline, the associations found between *COMT* val158met and 5HTTLPR and psychosis may be indirect via more rapid cognitive decline which may contribute to the emergence of the symptoms.

With regard to the polymorphisms specifically, there is a good body of literature linking *COMT* val158met to cognition, rate of cognitive decline and physiological responses associated with cognition. In particular, the val/val genotype has been associated with more rapid cognitive decline in two large studies, one of cognitively normal adults and one in 22q11.2 deletion syndrome (de Frias, Annerbrink et al. 2004, Gothelf, Eliez et al. 2005). The evidence surrounding 5HTTLPR is not so extensive and that which exists does not support a relationship (Payton, Gibbons et al. 2005). Nevertheless, in order to elucidate fully the role of these polymorphisms in psychosis it is essential that their impact on other biologically plausible aspects of the clinical syndrome is investigated. Cognitive decline is the most notable of these with regard to dementia. The results that follow are therefore an investigation of the association between the *COMT* val158met and 5HTTLPR polymorphisms and rate of cognitive decline in AD with the aim of further clarifying the role of these two polymorphisms in psychotic symptoms in AD.

4.1.1 Hypotheses

- The *COMT* val/val genotype will be associated with a faster average annual cognitive decline than val/met and met/met genotypes.
- It is not expected that the 5HTTLPR polymorphism will impact upon cognitive decline.
- Persistent psychotic symptoms will be associated with a faster rate of cognitive decline.

4.2 Methods

4.2.1 Software

Excel: data cleaning, coding and organisation.

STATA: data analysis and coding.

4.2.2 Inclusion/exclusion criteria

Inclusion criteria:

- MMSE conducted at baseline and at least one time point after 6 months or more, dates available for both MMSE assessments.
- Clinical or pathological diagnosis of possible, probable or definite Alzheimer's disease.
- Baseline MMSE greater than 9.
- Medication information relating to anti-dementia drug and antipsychotic exposure available.
- Serial assessment of psychotic symptoms available, meeting the criteria set out in section 3.3.2. Briefly, at least two assessments separated by six months or more.

Exclusion criteria:

- History of a psychotic disorder not related to dementia.

4.2.3 Design and cohort

COMT val158met and 5HTTLPR genotypes were available from 237 patients meeting the inclusion criteria specified in section 4.2.2 (see section 2.6 for genotyping methods). Patients were drawn from eight cohorts (ANM, ARUK, DCR, DEMVEST, Greek, MAIN-AD, Norway and OPTIMA).

4.2.4 Calculating cognitive decline

Cognition was evaluated using the MMSE and only those with a baseline score of 9 or more were included to avoid floor effects. An estimation of annual change in MMSE score was calculated using the baseline and last available scores (or the first point at which MMSE reached 0 provided it was 6 months or more after the first assessment) in three steps:

1) The date of the last MMSE assessment was subtracted from the date of the first to give the time elapsed between the two (in years).
2) MMSE score at last assessment subtracted from baseline MMSE score to give the change in number of points over the time period calculated above.
3) The number calculated in part 2) was then divided by the time elapsed calculated in part 1) to give an estimate of the change in MMSE score each year.

4.2.5 Statistical Evaluation

Relationships between annual MMSE decline and other clinical and demographic variables were assessed using Pearson's correlations, t-tests and one-way ANOVA as appropriate, any significant associations were controlled for in the main analysis. Of particular interest was the relationship between psychotic symptoms and cognitive decline. However because of the relatively low number of people with persistent symptoms, this relationship and its association with both polymorphisms was not statistically tested to keep comparisons to a minimum.

The primary analysis consisted of cases with a baseline MMSE greater than 9. A comparison between mean annual MMSE decline across the three genotypes of each polymorphism was conducted using ANOVA. Post-hoc analyses compared genotypes in the event of a significant ANOVA. The sample was then stratified by dementia severity, creating two groups: mild dementia and moderate dementia (defined by MMSE>19 and between 10 and 19 respectively) in order to examine whether the effect of genotype differs by dementia severity. Finally, a multivariate ANOVA was run to test the interaction between genotypes and dementia severity.

4.2.5.1 Power calculation

Assuming a MAF of 0.45 and an additive genetic model this sample size would have >80% power to detect 4% of the variance associated with rate of cognitive decline and approximately 60% power to detect 2% of the variance.

4.3 Results

4.3.1 Descriptive statistics: *COMT* val158met and 5HTTLPR

This sample consisted of 32 patients with pathologically confirmed possible, probable or definite Alzheimer's disease and 205 cases with clinically diagnosed possible or probable Alzheimer's disease.

Neither *COMT* val158met nor 5HTTLPR genotype differed significantly from HWE (*COMT*: val/met: χ^2=2.67, p>0.05; 5HTTLPR: χ^2=0.4, p>0.05).

Average annual MMSE decline for the group as a whole was 2.2 (SD±4.1). Neither age (r=-0.1, p=0.09) nor MMSE at baseline (r=0.09, p=0.2) correlated with rate of MMSE decline and mean annual decline did not differ by gender (mean difference: -0.4, t=-0.7, df=235, p=0.5). Likewise, average annual decline was not associated with persistent hallucinations (mean difference -0.4, t=-0.4, df=234,p=0.7) or delusions (mean difference: -1.4, t=-1.7, df=235, p=0.07) nor exposure to antipsychotics (mean difference: -0.4, t=-0.8, df=235, p=0.4) or anti-dementia drugs (mean difference: -0.55, t=-0.9, df=235, p=0.4) at any point during follow up.

Other participant characteristics by *COMT* val158met and 5HTTLPR genotypes are summarised in Table 4-1 and Table 4-2. None of the clinical or demographic variables listed differed significantly by *COMT* val158met genotype.

Table 4-1 Participant characteristics by study at baseline by *COMT* val158met genotype (N=237)

			***COMT* val158met genotype** [Freq(%)/Mean±SD]		
			val/val (n=56)	**val/met (n=131)**	**met/met (n=50)**
Age[1]			78 (7.2)	78 (7.7)	78 (6.6)
Gender[2]	Female		36 (62)	84 (64)	35 (69)
	Male		22 (38)	47 (36)	16 (31)
MMSE[3]			20 (5.1)	19 (5)	20 (5.2)
Mean follow up time (months)[4]			17 (12.6)	18 (15.2)	16 (13.4)
Anti dementia drugs[5]		No	38 (68)	99 (76)	37 (74)
		Yes	18 (32)	32 (24)	13 (26)
Antipsychotics[6]		No	31 (55)	74 (56)	28 (56)
		Yes	25 (45)	57 (44)	22 (44)

1. $F(2,234)=0.19$, p=0.8; **2.** $\chi^2=1$, df=2, p=0.6; **3.** $F(2,234)=0.39$, p=0.7; **4.** $F(2,234)=0.16$, p=0.9; **5.** $\chi^2=1.2$, df=2, p=0.5; **6.** $\chi^2=0.02$, df=2, p=0.9

With regard to 5HTTLPR, participant characteristics are summarised in Table 4-2. Again participants were well matched on each of the variables, with the exception of age where LS genotypes were significantly older (mean age 80 compared with 77 for LL and SS genotypes).

Table 4-2 Participant characteristics by study at baseline by 5HTTLPR genotype (N=237)

			5HTTLPR genotype [Freq(%)/Mean±SD]		
			LL (n=81)	**LS (n=111)**	**SS (n=45)**
Age[1]			77 (7.4)	80 (7.4)	77 (6.5)
Gender[2]	Female		53 (65)	69 (62)	31 (69)
	Male		28 (35)	42 (38)	14 (31)
MMSE[3]			20 (5.2)	19 (5.1)	20 (4.7)
Mean follow up time (months)[4]			17 (15.4)	17 (14.3)	17 (11.9)
Anti dementia drugs[5]		No	60 (74)	82 (74)	32 (71)
		Yes	21 (26)	29 (26)	13 (29)
Antipsychotics[6]		No	40 (49)	69 (62)	24 (53)
		Yes	41 (51)	42 (38)	21 (47)

1. $F(2,234)=4.3$, p=0.02; **2.** $\chi^2=0.7$, df=2, p=0.7; **3.** $F(2,234)=0.95$, p=0.4; **4.** $F(2,234)=0$, p=0.9; **5.** $\chi^2=1.2$, df=2, p=0.9; **6.** $\chi^2=3.3$, df=2, p=0.2

The remainder of the analysis is grouped by polymorphism, beginning with *COMT* val158met followed by 5HTTLPR.

4.3.2 *COMT* val158met and average annual MMSE decline

The first part of the analysis was descriptive, examining average annual decline by *COMT* val158met genotype and the presence/absence of persistent psychotic symptoms. This relationship was not statistically tested due to small numbers, the results are presented below. The differences between genotypes were similar for the delusions/no delusions groups and hallucinations/no hallucinations groups (Table 4-3 and Table 4-4 respectively). Overall, persistent delusions were associated with an annual MMSE decline of 3.4 points compared to 2 points among those without persistent delusions. For hallucinations there was no meaningful difference (symptom present: 2.1; no symptom: 2.3). With regard to *COMT* genotype, val/val carriers had an approximately 2-3.5 point higher annual decline than met/met carriers in each case.

Table 4-3 Average annual MMSE decline by COMT val158met and persistent delusions (N=237)

		Average Annual MMSE Decline [Mean ± SD]	
		Persistent delusions (n=51)	**No delusions (n=186)**
COMT val/met	Val/val	5 (4.2)	3.4 (3.2)
	Val/met	2.4 (5.1)	1.8 (4.1)
	Met/met	2.9 (5.3)	0.77 (3.2)

Table 4-4 Average annual MMSE decline by COMT val158met and persistent hallucinations (N=236)

		Average Annual MMSE Decline [Mean ± SD]	
		Persistent hallucinations (n=26)	**No hallucinations (n=210)**
COMT val/met	Val/val	3.8 (3)	3.7 (3.9)
	Val/met	2.1 (4.5)	1.8 (4.2)
	Met/met	0.25 (1.9)	1.3 (3.6)

The effect of *COMT* val158met genotype was first analysed on the total number of cases (N=237). The analysis was then extended to examine the same effect, with the sample stratified by dementia severity.

Average annual decline in MMSE for each *COMT* genotype group is depicted in Figure 4-1 and summarised in Table 4-5 ('total group' column). One-way ANOVA demonstrated a significant effect of *COMT* val158met genotype on average annual decline ($F(2,234)=5.92$, $p=0.003$). Post-hoc Bonferroni comparisons showed *COMT* val/val genotypes to have a significantly higher annual decline in MMSE than val/met and met/met genotypes ($p=0.005$ and $p=0.01$ respectively). Heterozygotes did not have a significantly faster rate of decline than met/met genotypes, although the mean for this group fell in between the other two.

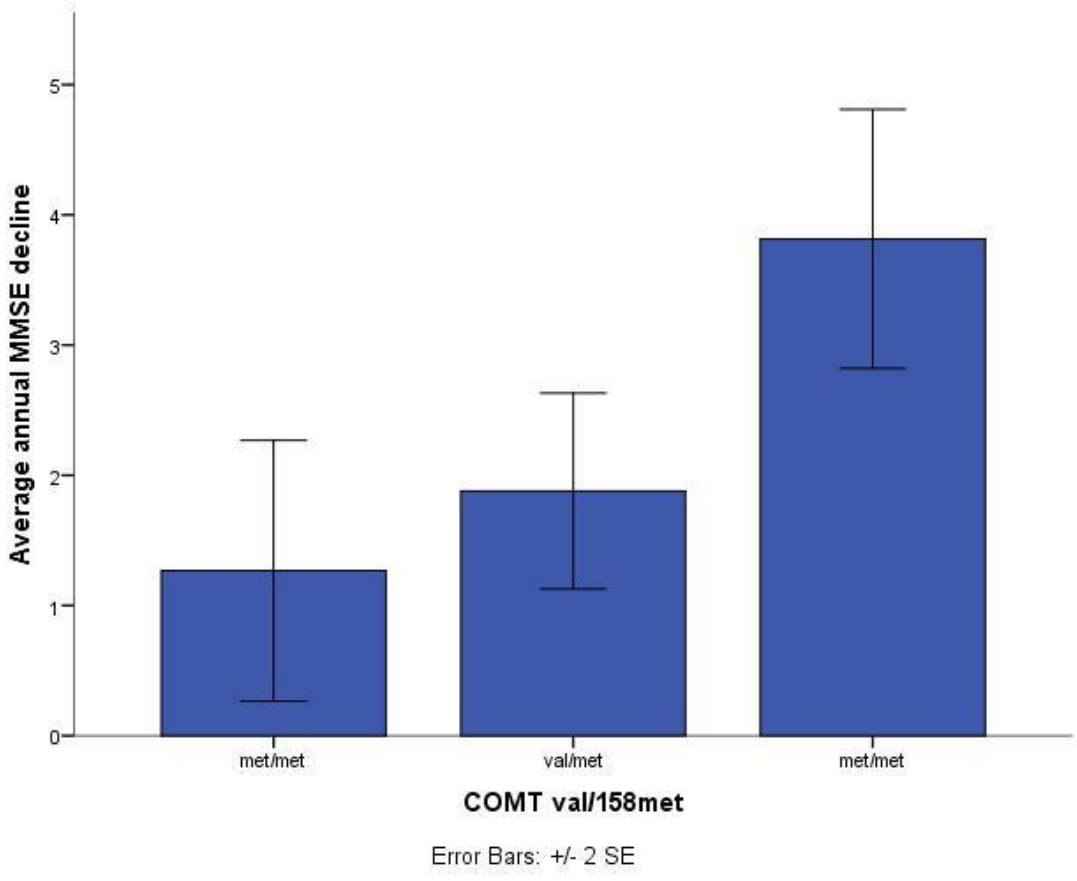

Figure 4-1 Average annual MMSE decline by *COMT* val158met genotype (N=237)

Table 4-5 Average annual MMSE decline and baseline MMSE score by *COMT* genotype (N=237)

		Baseline MMSE [Mean±SD]			**Average Annual MMSE Decline** [Mean±SD]		
		Moderate (n=112)	**Mild (n=125)**	**Total group**	**Moderate (n=112)[1]**	**Mild (n=125)[2]**	**Total group [3]**
COMT	val/val	15 (2.6)	24 (2.2)	20 (5.1)	3.2 (3.6)	4.2 (3.9)	3.8 (3.8)
	val/met	15 (2.8)	23 (2.4)	19 (5)	1.7 (4.6)	2.1 (4.1)	1.9 (4.3)
	met/met	15 (2.7)	24 (2.9)	20 (5.1)	1.7 (3.3)	0.8 (3.8)	1.3 (3.5)

1. $F(2,109)=1.38$, p=0.3; **2.** $F(2,122)=5.33$,p=0.006; **3.** $F(2,234)=5.92$, p=0.003

This effect of *COMT* was then examined by severity of AD. The analysis was stratified by MMSE, creating two groups, those with mild (20-30, n=125) and those with moderate AD (10-19, n=112), also summarised in Table 4-5 above and depicted in Figure 4-2 below. In the moderate group there was no difference in mean decline between val/val, val/met and met/met genotypes ($F(2,109)=1.38$, p=0.3). However, the association between *COMT* val158met and cognitive decline persisted in the mild group, with larger differences than those in the total group ($F(2,123)=5.82$,p=0.004). A post-hoc Bonferroni test in the mild group again showed val/val genotypes to be associated with a significantly higher average annual decline than met/met and val/met genotypes (p=0.007 and p=0.04 respectively). There continued to be no difference between the val/met and met/met groups. Finally, a multivariate ANOVA was run to test if any interaction between AD severity and *COMT* val158met genotype was present. Under this model, there was still a main effect of *COMT* ($F(2,231)=5.55$,p=0.004) but not of severity or the interaction.

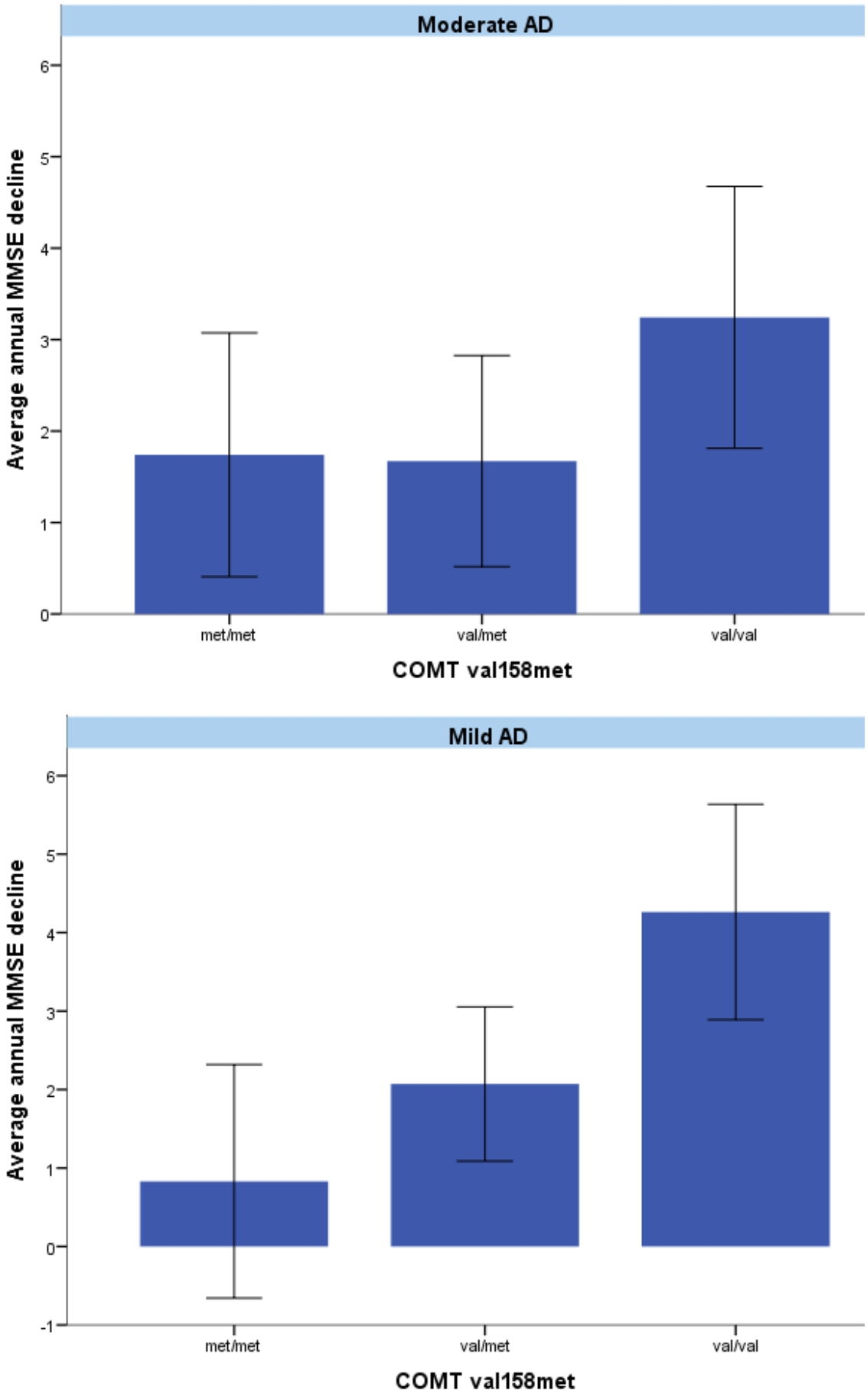

Figure 4-2 Average annual MMSE decline by COMT genotype and AD severity (N=237: moderate n=112; mild n=125)

4.3.2.1 Secondary analysis of *COMT* val158met including cases with severe AD

As a consequence of the finding that the effect of *COMT* val158met genotype on rate of cognitive decline does not appear to be present among those with moderate dementia a final exploratory analysis was carried out including cases with severe dementia (i.e. a baseline MMSE of less than 10 but greater than 4 in order to still provide some control over floor effects). Widening the inclusion criteria to include the individuals with severe AD resulted in another 29 patients in the analysis. Figure 4-3 shows average annual MMSE decline for all AD severities. Extending the analysis to include more severe cases as well as those in the less severe stages resulted in a weak but significant correlation between age and annual decline (r=-0.13, p=0.04), therefore age was included as a covariate in this ANOVA model. *COMT* val/val genotypes declined an average of 3.2 points per year on the MMSE compared with 2.3 and 1.3 for val/met and met/met genotypes respectively and this difference was found to be significant (F(2,262)=3.74, p=0.03).

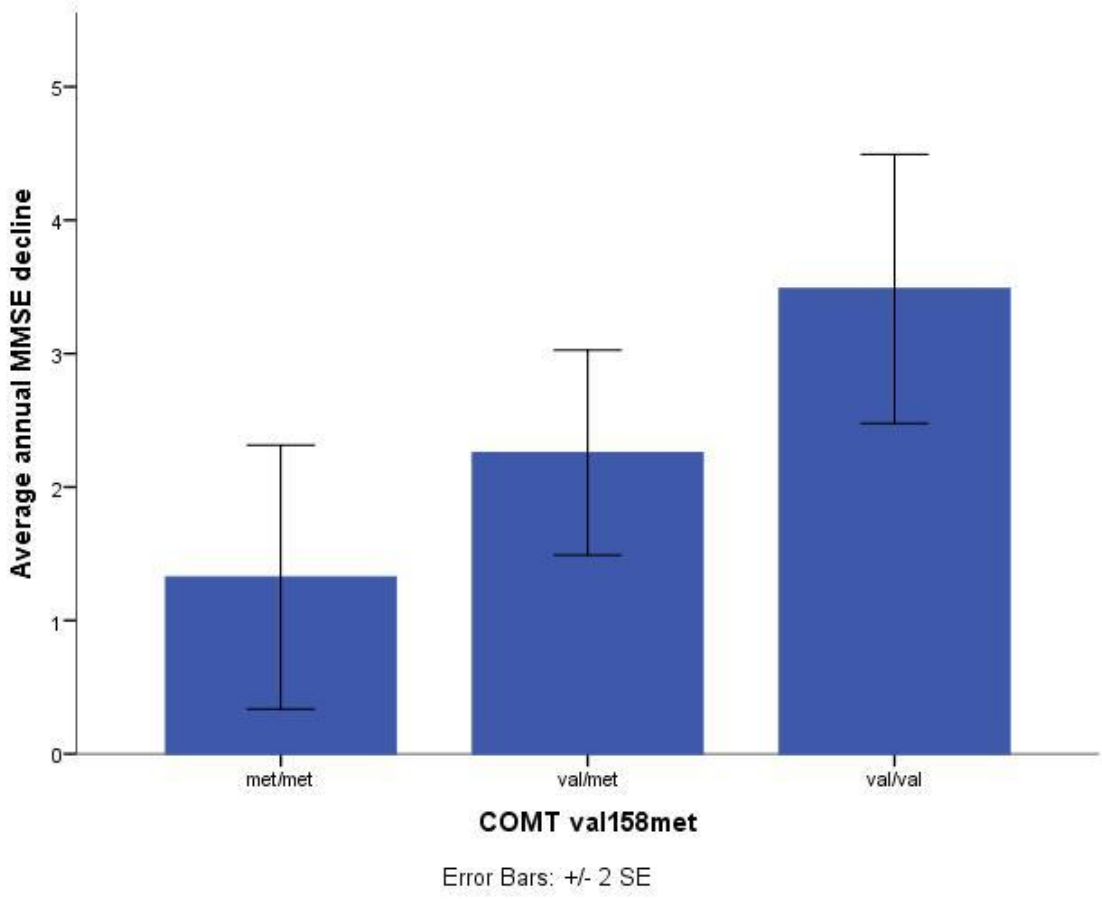

Figure 4-3 Average annual MMSE decline by *COMT* genotype, including severe cases (N=266)

Examination of the severe AD group only (n=29) was restricted to descriptive statistics only as there was only one case with the met/met genotype in this group. Average annual MMSE decline was 4.2, 4.7 and 1.1 for the met/met, val/met and val/val groups respectively. Although this relationship cannot be tested, it is worth noting that the val/val genotypes had the lowest rate of annual decline, whereas the reverse was true in the mild group.

4.3.3 5HTTLPR and average annual MMSE decline

The analysis for 5HTTLPR followed the same plan as *COMT* described above.

Descriptive statistics for average annual decline by genotype and symptom presence are summarised in Table 4-6 and Table 4-7, again these differences were not statistically tested. Regarding delusions, the biggest numerical difference was between the LL genotype (average annual decline 4.5) and the other genotypes (LS: 2.3, SS: 2.6) among those with persistent delusions, while the rate of decline was similar among those without persistent delusions.

With regard to hallucinations, the biggest difference was again in the persistent symptom group with SS genotypes having a rate of 5.8 compared to 2.5 and 0.46 in the LS and SS groups respectively. Again, in the no symptom group the rate of decline between genotypes was similar.

Table 4-6 Average annual MMSE decline by 5HTTLPR and persistent delusions (N=237)

		Average Annual MMSE Decline [Mean ± SD]	
		Persistent delusions (n=51)	**No delusions (n=186)**
5HTTLPR	LL	4.5 (5.3)	2.1 (4.3)
	LS	2.3 (4.4)	1.7 (3.4)
	SS	2.6 (5.6)	2.1 (3.9)

Table 4-7 Average annual MMSE decline by 5HTTLPR and persistent hallucinations (N=236)

		Average Annual MMSE Decline [Mean ± SD]	
		Persistent hallucinations (n=26)	**No hallucinations (n=210)**
5HTTLPR	LL	0.46 (4.5)	2.8 (4.6)
	LS	2.5 (3.4)	1.6 (3.5)
	SS	5.8 (3.1)	1.9 (4.2)

Average annual decline in MMSE for each 5HTTLPR genotype in the total group is shown in Figure 4-4, and in the right-most column of Table 4-8. There was no association between average annual decline and 5HTTLPR genotype in the total group (*F*(2,234), p=0.4,Table 4-8).

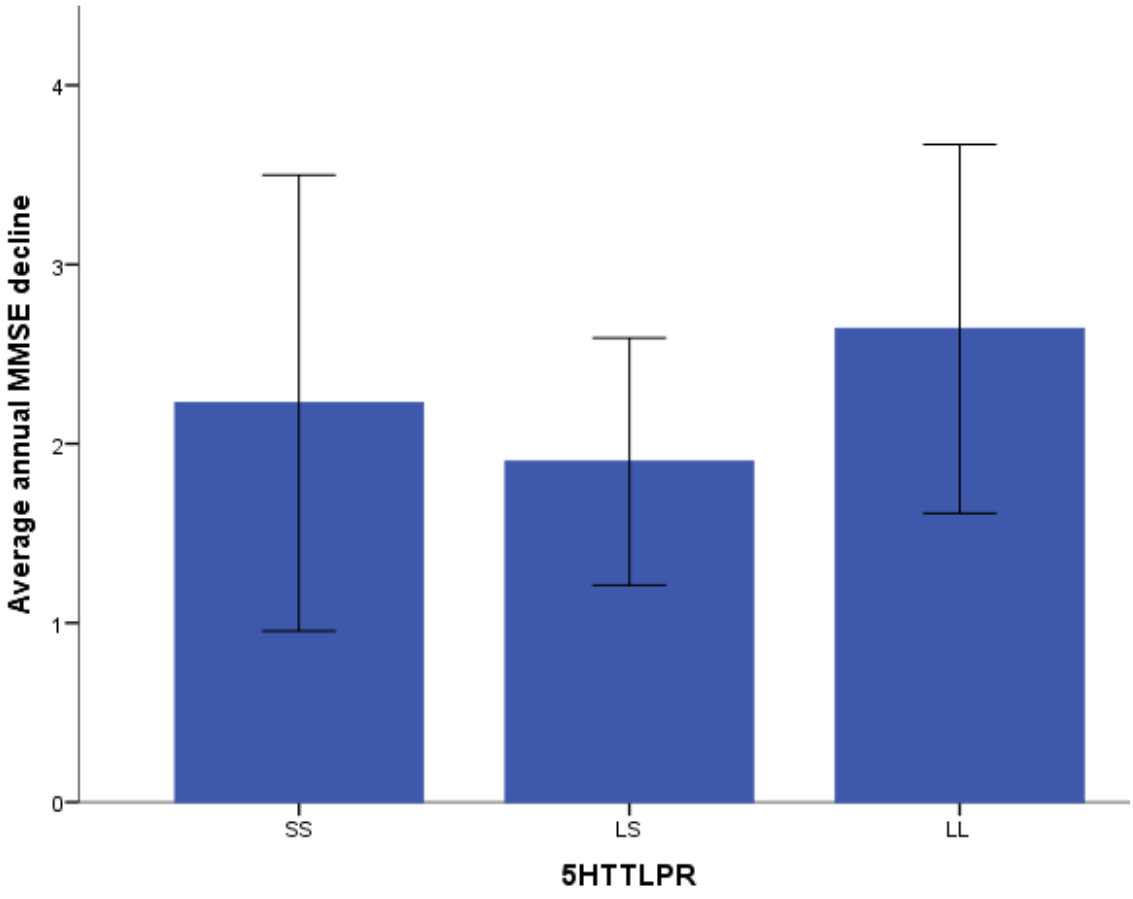

Figure 4-4 Average annual MMSE decline by 5HTTLPR genotype (N=237)

Table 4-8 Average annual MMSE decline and baseline MMSE score by 5HTTLPR genotype (N=237)

		Baseline MMSE [Mean±SD]			**Average Annual MMSE Decline** [Mean±SD]		
		Moderate (n=112)	**Mild (n=125)**	**Total group**	**Moderate (n=112)[1]**	**Mild (n=125)[2]**	**Total group [3]**
5HTTLPR	LL	15 (2.6)	24 (2.9)	20 (5.2)	2.7 (3.6)	2.6 (5.3)	2.6 (4.6)
	LS	15 (2.8)	24 (2.2)	19 (5.1)	1.7 (4.1)	2.1 (3.1)	1.9 (3.6)
	SS	15 (2.7)	24 (2.3)	20 (4.7)	2 (5.2)	2.4 (3.5)	2.2 (4.3)

1. ANOVA: $F(2,109)=0.63$, p=0.5; **2.** ANOVA: $F(2,122)=0.23$, p=0.8; **3.** ANOVA: $F(2,234)=0.86$, p=0.4

Table 4-8 above and Figure 4-5 below show average annual MMSE decline by 5HTTLPR genotype stratified by AD severity. There remained no significant effect of genotype on annual decline in participants with moderate ($F(2,109)=0.63$, p=0.5) and mild ($F(2,122)=0.23$, p=0.8) AD. There was also no significant interaction between AD severity and 5HTTLPR genotype.

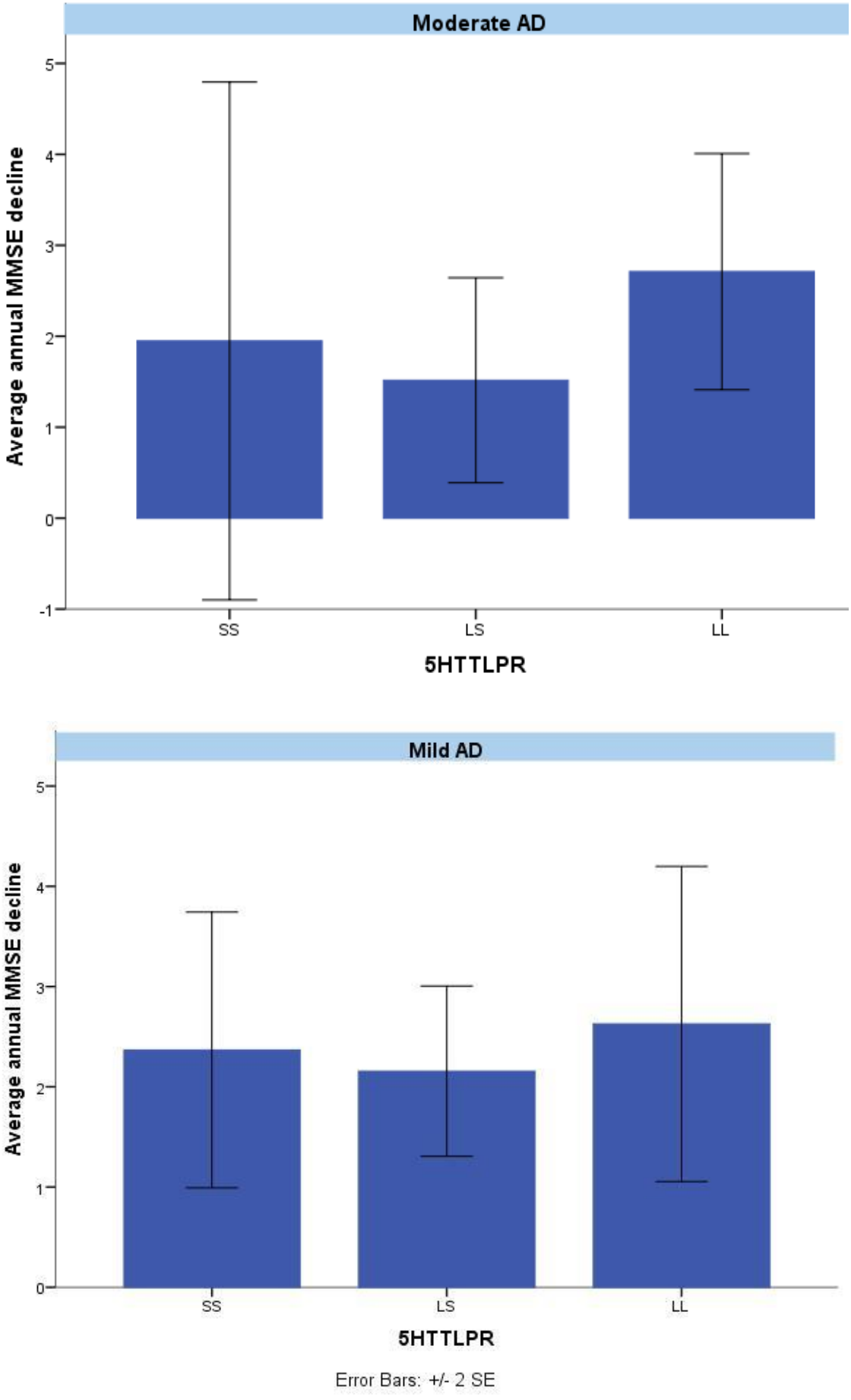

Figure 4-5 Average annual MMSE decline by 5HTTLPR genotype and dementia severity (N=237: moderate n=112; mild n=125)

4.3.3.1 Secondary analysis of 5HTTLPR including cases with severe AD

The analysis was extended to include those with an MMSE below 10 and above 4. Figure 4-6 shows average annual MMSE decline for all dementia severities. Age was again included as a covariate due to the weak but significant correlation (r=-0.13, p=0.04). There was no significant difference in annual decline between 5HTTLPR genotypes (F(2,262)=0.56, p=0.6).

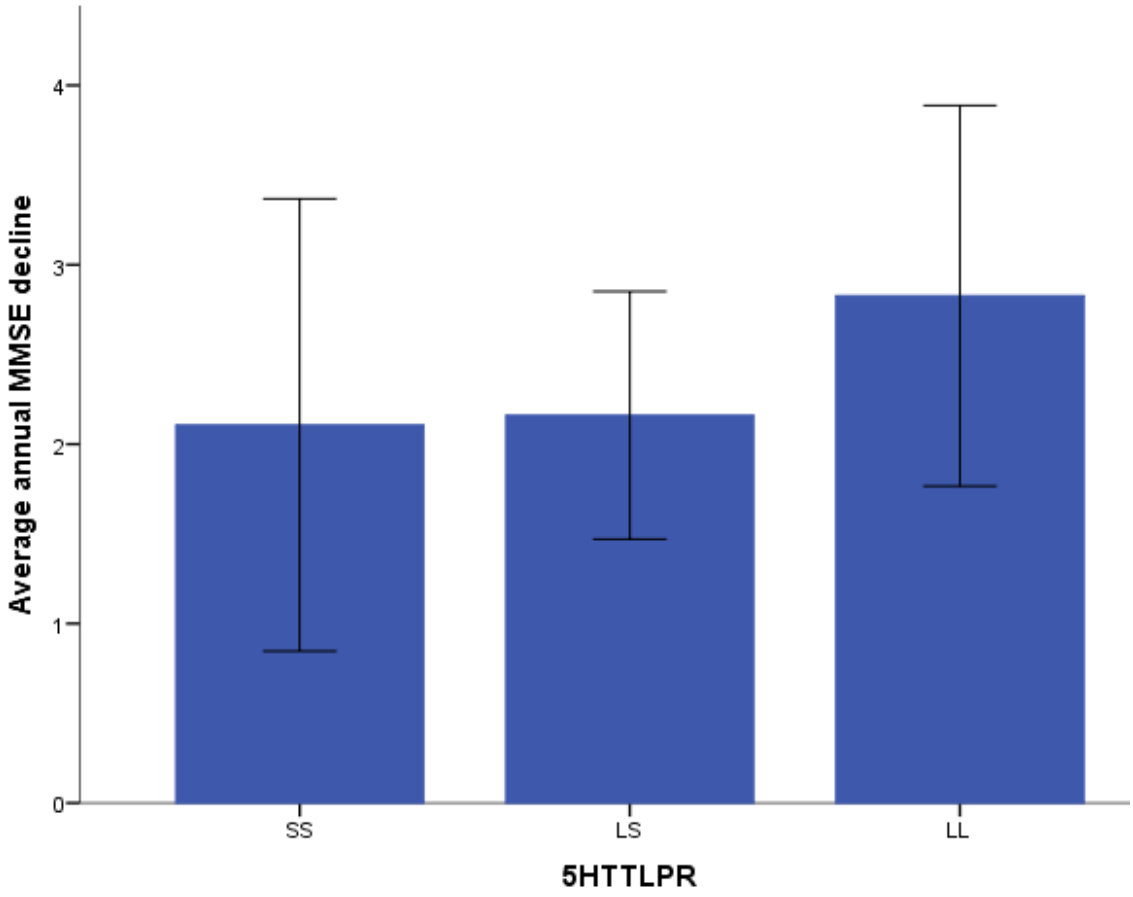

Figure 4-6 Average annual MMSE decline by 5HTTLPR genotype including severe cases (N=266)

Examination of the severe dementia group only (n=29) was restricted to descriptive statistics as there were only two cases with the SS genotype in this group. Average annual MMSE decline was 4.3, 3.9 and -0.6 (i.e. a very small improvement) for the LL, LS and SS groups respectively. Again, although not statistically tested, SS genotypes did have a lower rate of decline than the other groups, whereas the LS genotypes had the lowest rate of decline in the mild and moderate groups.

4.4 Summary of findings

4.4.1 Summary of primary analyses

1. *COMT* val/val genotype is associated with a higher average annual MMSE decline than val/met and met/met genotypes (mean annual decline: 3.8, 1.9 and 1.3 respectively).
2. This finding was particularly pronounced in people with Mild AD (val/val: 4.2; val/met: 2.1; met/met: 0.8) but not in moderate cases in an analysis stratified by dementia severity.
3. There was no association between 5HTTLPR genotype and average annual MMSE decline.

4.4.2 Summary of secondary analyses

1. The inclusion of participants with MMSE<10 but >4 did weaken the effect of *COMT* val158met genotype, however it was still significant (val/val: 3.4; val/met: 2.3; met/met: 1.3, p=0.03).
2. The addition of the severe cases made no difference to the impact of 5HTTLPR upon cognitive decline: there was still no significant association.

4.5 Discussion

In the 237 individuals with mild to moderate AD the *COMT* val158met polymorphism was significantly associated with average annual MMSE decline: val/val carriers declined at a rate of 3.8 points per year, compared with 1.9 and 1.3 for val/met and met/met carriers respectively. Subsequent planned analysis demonstrated that this difference was mostly driven by those with mild AD, in whom the difference was particularly pronounced. In this latter stratified analysis there was no significant difference in decline among the moderate AD group, however the differences became more pronounced in the mild AD group with the val/val carriers having an average decline of 4.2, compared with 0.8 among met/met carriers. Widening the inclusion criteria to include 29 patients with an MMSE >4 weakened the effect of the *COMT* val158met genotype, although val/val carriers still had a significantly faster rate of decline (p=0.03). Although nominally significant, not meeting a more stringent significance threshold adjusted for multiple comparisons, this provides further support that this effect is restricted to people with mild AD. Moreover in a descriptive analysis the severe AD results are the reverse of mild, though this needs statistical confirmation. The 5HTTLPR polymorphism was not found to effect rate of decline in this AD cohort. These findings are therefore in line with the bulk of the non-AD literature suggesting that *COMT* is a key mediator of cognitive function, while the evidence around 5HTTLPR is much weaker.

Every patient in this sample was included in the AD results presented in Chapter 3; it was therefore assumed that there was no association between each polymorphism and psychosis. In the case of *COMT* val158met, the suggestion here is that some of its observed association with psychosis in previous studies may be indirect via an association with more rapid cognitive decline and this could explain some of the variability in the results (Sweet, Devlin et al. 2005, Borroni, Grassi et al. 2006, Proitsi, Lupton et al. 2010).

The average annual decline of people with persistent delusions was 1.2 points higher than those without. Although not statistically significant (p=0.07), in light of it being consistent with previous reports it is worth noting (see section 4.3.1) (McShane, Keene et al. 1997, Drevets, Rubin 1989, Paulsen, Salmon et al. 2000). The McShane et al. (1997) study only found persecutory delusions to be associated with more rapid cognitive decline. Not being able to distinguish these from other

types of delusions may have masked an effect in the present study. However the other two studies both examined psychosis as a composite of both delusions and hallucinations and still reported a significant effect. The rating of psychosis in the present study was captured over a period of at least six months and the calculation of cognitive decline would not necessarily be based on the same period of time (that is, it could be based on a period before the emergence of symptoms, or after, or both). However, based on previous results an association with more rapid cognitive decline would still be expected because it has been shown to precede the onset of psychosis (Paulsen, Salmon et al. 2000). None of the aforementioned studies employed the Folstein (the original) MMSE as a measure of change, as was the case in this study (a full description of the MMSE is included in section 2.2.3.1). McShane et al. (1997) used a modified version of the MMSE, which includes additional items relating to recent episodic memory, short term recall and language. The Mattis Dementia Rating Scale (as well as other tests of executive function) which was employed by Paulsen et al. focuses less on language and more on conceptualisation and construction, while memory and attention are still covered. In contrast, The Blessed Dementia Rating Scale focuses very heavily on memory, with a small section on concentration. The fact that three different rating scales have been used and all have found an association between psychosis and rate of cognitive decline could be taken as indicating quite a robust association, which would make it all the more puzzling why no effect was found here. However, it could be that the relatively greater focus on language in the original MMSE makes it less sensitive to the specific cognitive domains associated with psychosis, which at present remain to be elucidated but may be those related to executive functioning (Paulsen, Salmon et al. 2000).

These points notwithstanding the results here highlight another important dimension in genetic association studies of psychosis in AD. It has been noted earlier in this thesis (Creese, Ballard et al. 2013) that cognitive impairment, by virtue of being a correlate of psychosis in AD, may have confounded previous studies on 5HTTLPR and the arguments made in that section are still relevant to *COMT* val158met. Although there is no evidence to suggest 5HTTLPR is associated with more rapid cognitive decline in this study, the *COMT* val158met association may be an important source of the variance in the psychotic phenotype in AD attributable to genetic variation. To evaluate this thoroughly would require an extremely well characterised cohort and analysis of the *COMT* val158met polymorphism in individuals with and without psychotic symptoms.

Although the present cohort is characterised in this way, there were only 51 (22%) cases with persistent delusions and 26 (11%) with persistent hallucinations, the decision was therefore taken not to undertake statistical comparisons in view of the low power and to restrict the number of hypotheses being tested. However, it is worth noting that there was a modest numerical difference in average annual decline between *COMT* val/val genotypes with and without persistent delusions and there was a much larger difference relating to 5HTTLPR. LL genotypes declined two points per year more than SS genotypes among those with persistent delusions, and SS genotypes declined over five points more per year that LL genotypes among those with persistent hallucinations. In the no symptom groups average annual decline was approximately two for all 5HTTLPR genotypes. No conclusions can be reliably drawn about this relationship until it has been statistically examined in much larger samples. However, in an area of literature where the relationship between 5HTTLPR and psychosis in AD is still inconclusive it would be prudent to consider interactions with cognitive decline to explain the relationship fully.

The finding presented here relating to the *COMT* val158met polymorphism is in line with the previous literature in cognitively normal adults. Two out of three studies of longitudinal cognitive decline have reported that the val/val genotype is associated with more rapid decline in several different domains (de Frias, Annerbrink et al. 2005, Starr, Fox et al. 2007, de Frias, Annerbrink et al. 2004). The implication from the findings presented here is therefore that reduced levels of DA in the prefrontal cortex (PFC) are not only detrimental to cognition over time in control participants but also in AD. However, *COMT* is not likely to be a risk factor for AD itself (Harold, Abraham et al. 2013). The association in healthy controls is therefore not necessarily pathological; indeed the effects observed in these groups are not of a size such that it would interfere with daily functioning. The annual decline observed in the mild group in this study however is clinically meaningful: 4.2 for val/val carriers compared to 0.8 against a baseline MMSE of 24. It might therefore be hypothesised that the decreased PFC DA levels associated with the val/val genotype are particularly detrimental to cognition once dementia is present. The exact reason for this heightened sensitivity to the val/val genotype in AD is not known, but evidence from neuroimaging studies may provide some insight into the anatomical phenotype that may underlie this observed cognitive one.

In a study of grey matter volume associated with the *COMT* val158met polymorphism, Gennatas et al. (2012) found that the val/val genotype was associated with a statistically significant decrease in a combined cohort of 169 dementia patients (77 with AD) and 83 controls (Gennatas, Cholfin et al. 2012). Stratification by disease status led the authors to conclude that this effect was being driven mostly by the patient group. The authors did not examine cognitive decline in their cohort, and although the *COMT* val158met polymorphism was not associated with level of cognitive impairment it is possible that the decline observed in the present study is a clinically observable result of the increased grey matter loss associated with the val/val genotype reported by Gennatas et al. The dementia participants in the Gennatas et al. study were overall mildly impaired (mean MMSE 21). Taken together with the results presented here, identifying *COMT* val/val individuals may therefore prove a useful clinical trial enrichment strategy. If val/val genotypes represent a sub group with a 4-fold higher rate of decline in mild AD compared to met/met genotypes this would be the optimum population from which to draw RCT participants as the faster decline would give much greater statistical power. More specifically, one class of drug where this application might be beneficial is in the use of *COMT* inhibitors. Under this hypothesis, the rapid cognitive decline in val/val genotypes would be due to DA deficiency in the PFC caused by the relatively higher activity of this genotype, which could be mitigated by inhibition of COMT. Clearly, much more work needs to be conducted before this hypothesis can be tested in AD, not least the initial step of reliable replication of the present results which is most important at this stage.

Although an overall effect of the *COMT* polymorphism was observed in the sample presented here as a whole, the results in this study were driven by the mild dementia group. Although there was a numerical increase in decline among the val/val genotypes compared to heterozygotes and met/met genotypes in the moderate group, this failed to reach significance. Both severity groups had a similar average annual decline (moderate 2.2; mild 2.3), ruling out the possibility that rate of decline in moderate dementia increases to an extent that the variance explained by the *COMT* val158met polymorphism becomes too small to detect with the sample size. It is, however, worth considering whether the decline in the type of cognitive domain may change across the course of AD. The influence of *COMT* val158met on the full range of cognitive domains remains to be elucidated but decline in both declarative memory (in particular recall) and executive functioning have been associated with the val/val genotype (de Frias, Annerbrink et al. 2004, de Frias,

Annerbrink et al. 2005). In the case of the de Frias et al. (2004) study, declarative memory specifically referred to performance on memory recall tests. In the case of the latter 2005 study executive function, specifically working memory and planning, was tested in a composite measure. There are aspects of working memory and recall in the registration and recall sections of the MMSE as well as the attention/calculation section. In the case of the former, three words have to be encoded then held for a short period of time before being repeated and in the case of the latter the word "world" has to be retained in memory in order to be able to spell it backwards. Recent recall and short term memory are two of the domains that are affected particularly early in the course of AD (Forstl, Kurz 1999, Storandt 2008). It therefore may be possible that the observed association with rate of decline in cognition and *COMT* val158met genotype in mild AD cases reflects the effect of COMT on these domains as measured by the MMSE. While in the moderate stages the decline principally takes places in domains such as language (comprehension and expression) (Forstl, Kurz 1999), which may be less likely to be associated with the *COMT* val158met polymorphism, but still captured by the MMSE, hence no effect is observed. Another avenue for future research would therefore be to examine the effect of the *COMT* val158met polymorphism on tests specific to executive functioning, or alternatively to determine whether indeed it is specific sub-scale scores of the MMSE that are associated with the *COMT* val158met polymorphism in different stages of dementia. The average annual MMSE decline for the severe group in this study was not tested due to there being only one met/met genotype among the 29 cases in this category. It would be interesting for future research to examine whether there is also no relationship with *COMT* and cognitive decline in people with severe AD. It is hypothesised here that there would be no such relationship as the main area of decline at this stage would be language and perhaps long-term episodic memory which are not domains typically associated with COMT functioning.

The same observation could be applied to the finding regarding 5HTTLPR. Although there is no support for 5HTTLPR being associated with more rapid cognitive decline, in keeping with the only other previous piece of published research in this area (Payton, Gibbons et al. 2005), it cannot be excluded that 5HTTLPR may be related to other cognitive functions not captured by the MMSE.

It is important to note that the wider literature is still not completely conclusive about the role of *COMT* val158met in cognition. This is illustrated in two meta-analyses that have been conducted

in this area examining performance on different tests, one reporting an association and the other reporting no association (Barnett, Jones et al. 2007, Barnett, Scoriels et al. 2008). However, this is offset somewhat by the stronger evidence of PFC function in cognition, the high expression of *COMT* in this brain region and the knowledge of the functional consequences of the *COMT* val158met polymorphism (Robbins, Arnsten 2009, Akil, Kolachana et al. 2003, Williams-Gray, Hampshire et al. 2007). This leads on to one of the primary caveats of interpretation on this study, which has already been noted above: replication. Overall, the strengths of this study include the good characterisation of relevant medication (anti-dementia drugs and antipsychotics) and the presence of psychotic symptoms, both of which have been associated with improvements or worsening of cognition. This therefore gives greater confidence that the observations noted in this study are the result of genetic association. Even so, this is the first study examining the role of the *COMT* val158met polymorphism in cognitive decline in AD and therefore it will be important for this result to be independently replicated before the conclusions presented can be widely accepted.

More specific to this study, it must be acknowledged that annual cognitive decline was an approximation (the first and last recorded MMSE divided by the number of years followed up). Ideally, this study should be repeated in a sample where MMSE is measured at the start and end of a year, or every year for a set number of years. This would allow an exact decline to be calculated and for MMSE to be treated as a within-subjects variable in a repeated measures design, which is perhaps the most appropriate form of analysis for this type of study. Taking either of these approaches would have been possible in the present study however it would have severely limited the sample size. Following the decision to use an approximation, descriptive statistics were examined, which indicated that the estimated of decline of 2.2 points per year on the MMSE generated here is a reasonable one. Firstly because it is broadly similar to previous estimates, with most studies placing annual MMSE decline between 2 and 4 points (Holmes, Ballard et al. 2005, Ballard, O'Brien et al. 2001, Roselli, Tartaglione et al. 2009, McCarten, Rottunda et al. 2004). Secondly it was based on a mean length of follow up of 17 months, with a lower cut off of 6 months or more. Moreover, the vast majority of cases were followed up for not more than three years (mean follow up 17 months SD±14.2) indicating that the approximation to one year was based on figures close to that.

In summary, this study presents the first evidence that the *COMT* val/val genotype is associated with more rapid cognitive decline in AD, suggesting reduced DA in the PFC may be an important target for treatment of cognitive decline among individuals with this genotype. Furthermore, it also brings greater clarity to current understanding of the role of *COMT* val158met in the wider AD psychosis literature by suggesting an indirect association with psychosis may be present via more rapid cognitive decline.

Chapter 5 The extended *MAPT* haplotype and worsening of psychotic symptoms in Alzheimer's disease

5.1 Summary introduction

There is a solid body of research linking neurofibrilliary tangle (NFT) pathology with the presence of psychotic symptoms in people with AD (Farber, Rubin et al. 2000, Zubenko, Moossy et al. 1991, Forstl, Burns et al. 1994, Murray, Kirkwood et al. 2013), although not universally (Sweet, Hamilton et al. 2000). A finding common to three of these studies is increased NFT burden in the frontal cortex, with the entorhinal cortex also being implicated in two studies, suggesting frontal regions or possibly hippocampal-neocortical pathways may be implicated in the pathogenesis of psychotic symptoms in AD.

NFT pathology is one of the hallmark features of AD and understanding its role in the disease course will in turn further understanding of the place of psychotic symptoms in people with AD. Sweet et al. (2003) have proposed that AD with psychosis constitutes a distinct phenotype (Sweet, Nimgaonkar et al. 2003). It is hypothesised that disease modification genes may exist that are only a risk for psychosis in the context of AD (the disease modification pathway) and also that there may be genes that predispose individuals to develop AD with psychotic symptoms (the heterogeneity pathway). Therefore to fully understand the genetic architecture of psychotic symptoms in AD, putative AD risk genes should be investigated. Given the aforementioned pathological findings concerning NFT burden and psychosis, the *MAPT* gene, which codes for the tau protein, is a good candidate. A further advantage of a genetic association approach in this context is that it is possible to examine individuals over a period of time. While pathological studies have indicated that NFT burden is associated with psychosis, not all have been able to address the concern of dementia severity as a confounding variable (Forstl, Burns et al. 1994, Zubenko, Moossy et al. 1991). Therefore establishing the true correlate of psychosis (NFT burden itself or an indirect association with disease severity) is difficult. With a genetic association approach individuals can be examined longitudinally and the course of symptoms evaluated with respect to genotype, something that is impossible in pathological studies.

The extended haplotype of *MAPT* has previously been associated with AD and other tauopathies, although not consistently. It has been proposed that upregulation of the 4R isoform, which has a greater propensity to aggregate into NFTs, resulting from carriage of the H1c variant of the H1 haplotype (which is suggested to drive the previous associations between H1 and tauopathies) is

in part the reason for this increased risk (Myers, Pittman et al. 2007). There has only been one previous report of an investigation into the *MAPT* gene and psychosis in AD, which proved to be negative (DeMichele-Sweet, Klei et al. 2011). However, although participants were well characterised for the presence of symptoms this was a prevalence study and did not take into account the longitudinal course of symptoms, something which is important in establishing whether some genes adversely affect disease course.

The clinical trials from which patients for this study were drawn (MAIN-AD and MAGD) were examining memantine as a treatment for neuropsychiatric symptoms in patients with AD living in care homes. Memantine is an interesting candidate for treatment of psychosis given its putative role in inhibiting and reversing tau phosphorylation, however treatment with memantine for these symptoms has to date only demonstrated a very modest effect in RCTs (Wilcock, Ballard et al. 2008, Gauthier, Loft et al. 2008, Fox, Breitner et al. 2012). Although research into memantine and tau phosphorylation is in its very early stages a preliminary experiment was also undertaken to evaluate whether the extended *MAPT* haplotype modified response to memantine.

Thus, in the present study the aim was to examine the effect of the extended *MAPT* haplotype on the likelihood of delusions and hallucinations worsening or emerging during 12 weeks of follow up during the two RCTs. A second preliminary analysis sought to identify whether the extended *MAPT* haplotype modified memantine treatment response.

5.1.1 Hypotheses

- The *MAPT* haplotype will be associated with a worsening of psychotic symptoms.

5.2 Methods

5.2.1 Software

Excel: data cleaning, coding and organisation.

STATA: data analysis and coding.

5.2.2 Inclusion/exclusion criteria

Inclusion criteria:

- As per MAIN-AD and MAGD clinical trial protocols (see General Materials and Methods section), briefly:
    - Met NINCDS-ADRDA criteria for possible or probable AD.
    - Living in a care home.
    - Stable dose of psychotropic medication for at least 4 weeks prior to starting the trial.
    - Not taking medication which is contraindicated to memantine.
- Consent for DNA obtained and sample taken.
- Neuropsychiatric Inventory (NPI) and Functional Assessment Staging Tool (FAST) both recorded.

Exclusion criteria:

- As per MAIN-AD and MAGD clinical trial protocols (see General Materials and Methods for full description), briefly:
    - Taking memantine.
    - Previous history of intolerance to memantine or related drugs.
    - DMS-IV Axis I disorder other than in the context of AD.
    - Severe renal impairment.
- Consent for DNA declined.

5.2.3 Design and cohort

Ninety-five buccal swab samples from patients identified from the MAIN-AD and MAGD databases were genotyped for the extended *MAPT* haplotype and analysed by electrophoresis (see sections 2.4 and 2.6.3 for methods concerning DNA collection/extraction and genotyping respectively). Demographic data was available for all participants, baseline cognitive impairment was measured using the MMSE and dementia severity was measured using the FAST.

5.2.3.1 Memantine exposure and efficacy evaluation

As part of MAGD and MAIN-AD, participants were randomly assigned to receive either memantine or placebo (MAGD) or memantine or antipsychotic (MAIN-AD) in a 1:1 allocation. For the present analysis, participants were coded as either receiving memantine (1) or not (0).

The efficacy of treatment with memantine was evaluated separately for delusions and hallucinations over 12 weeks by measuring change in the NPI subscale scores. Baseline and 12 week symptom scores were calculated by multiplying the frequency and severity ratings. The 12 week score was then subtracted from the baseline score giving a figure either below zero (symptom worsened), zero (no change) or above zero (symptom improved). Participants were then classified into one of two treatment outcome groups: no change/ improved (0) or worsened (1), see section 5.2.4.1 for justification of this treatment.

5.2.4 Statistical Evaluation

5.2.4.1 Treatment of NPI subscale scores

The single symptom subscales of the NPI are non-parametric when analysed alone, and in the case of the present study so was the symptom change score (derived from subtracting the 12 week score from the baseline score). Summed variables describing syndromes identified by statistical techniques and comprising several NPI subscale scores are often used for analysis and are more likely to be normally distributed. However as noted in several previous sections, this approach is not felt to be appropriate for the analysis of the underlying biology of psychotic

symptoms due to evidence that that the aetiology of delusions and hallucinations is at least in part heterogeneous. In the current data set both log and square root transformation failed to correct the substantial deviation from normality. Therefore the decision to divide the scale into categories was taken. There were a substantial number of zeros in the data set therefore splitting into tertiles or about the median was not possible. The decision was therefore taken to dichotomise the sample based on the change in symptom scores. Examination of Figure 5-1 and Figure 5-2 below shows that a number of participants' symptoms worsened during the course of the study. Given that this is the least desirable clinical outcome for a patient, this group was compared to an aggregated group comprising those whose symptoms remained the same (a score of 0) and those whose symptoms improved (a score >0). This decision was also based upon the meta-analysis by Gauthier et al. (2008) which showed that significant difference in delusions scores after 12 weeks of memantine vs. placebo was driven by a worsening of the latter group, while the memantine group remained stable (Gauthier, Loft et al. 2008).

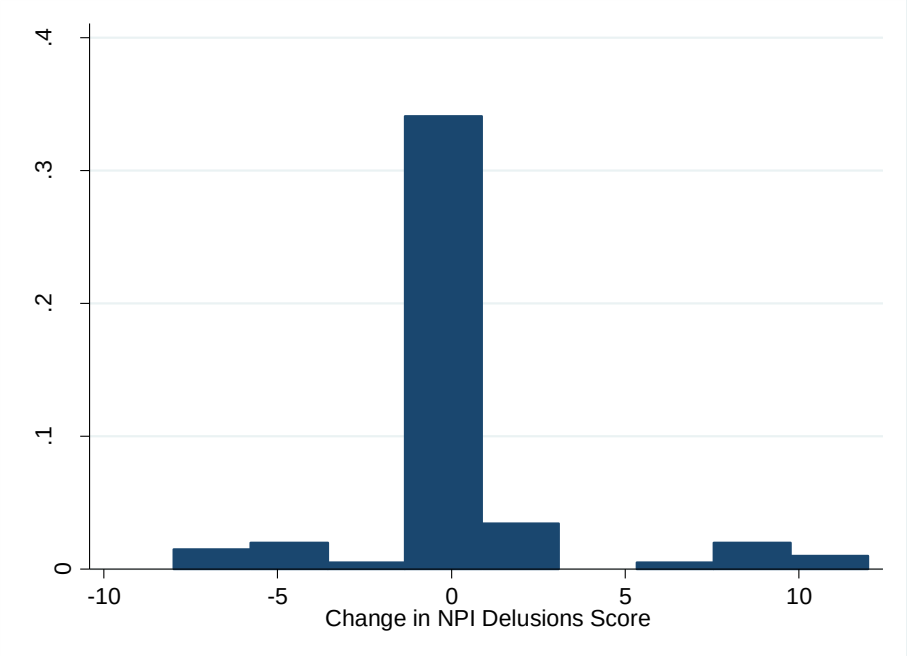

Figure 5-1 Histogram of change in NPI delusions score from baseline to 12 weeks

Lower than zero: worsening symptom. Higher than zero: improving symptom

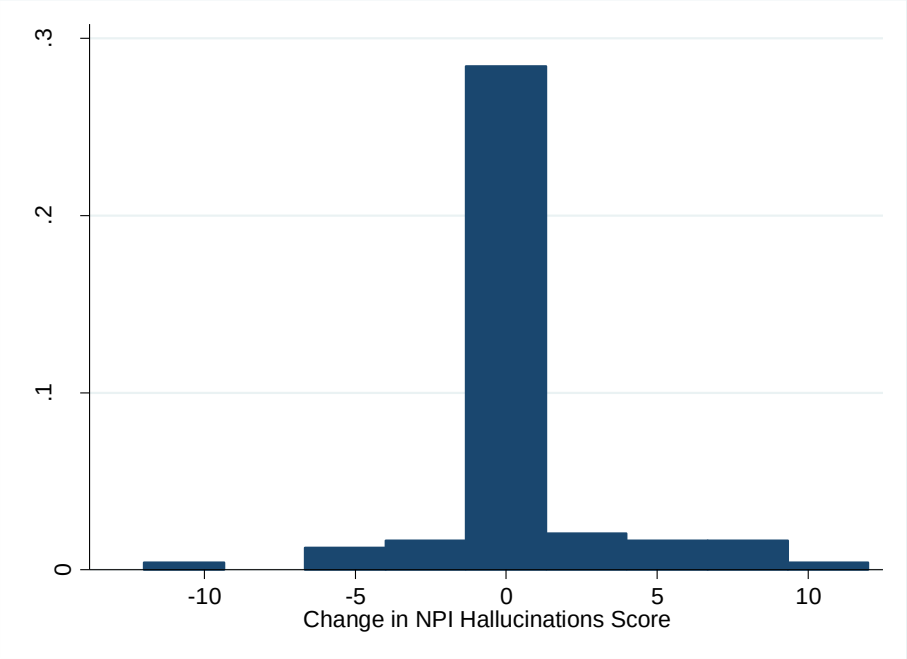

Figure 5-2 Histogram of change in NPI delusions score from baseline to 12 weeks
Lower than zero: worsening symptom. Higher than zero: improving symptom

5.2.4.2 Analysis

ANOVA, t-tests (or non-parametric equivalents) and chi square were used to compare means and frequencies of participant demographics respectively. Binary logistic regression models with treatment outcome as the dependent variable were used to generate odds ratios (ORs) for treatment arm, *MAPT* haplotype and other clinical and demographic variables. The sample was then stratified by memantine treatment arm and further logistic regression models were run on each stratum. Finally the interaction between treatment arm and *MAPT* haplotype was generated and included in the model.

5.2.4.3 Power calculation

This is an exploratory study on which to base future research, therefore a power calculation was not carried out.

5.3 Results

5.3.1 Descriptive statistics

Baseline MMSE score was only available for 64 participants, therefore when evaluating the impact of dementia severity the FAST rating was used. The one patient with a rating of 4 was grouped with the 5 rated group to create a mild/moderate group which was compared with the 6 and 7 rated groups (i.e. three groups in total).

Demographic variables were then examined by memantine treatment and *MAPT* haplotype; the results are presented in Table 5-1. There were no significant differences in mean values of frequencies across any of the treatment/haplotype group combinations. Due to the low frequency of *MAPT* H2/H2 carriers, these patients were analysed in combination with the heterozygote group, creating two groups (H1/H1 and H2*).

Table 5-1 Participants characteristics at baseline by treatment and *MAPT* haplotype (N=95)[1]

		No memantine (n=47) [Mean ±SD / Frequency (%)]			Memantine (n=48) [Mean ±SD / Frequency (%)]		
		***MAPT* H1/H1 (n=29)**	***MAPT* H1/H2 (n=14)**	***MAPT* H2/H2 (n=4)**	***MAPT* H1/H1 (n=33)**	***MAPT* H1/H2 (n=13)**	***MAPT* H2/H2 (n=2)**
Age[2]		84 (6.3)	84 (7.6)	81 (11)	84 (8.3)	79 (9)	80 (2)
Antipsychotic [3]	No	5 (17)	4 (29)	2 (50)	29 (88)	11 (85)	1 (50)
	Yes	24 (83)	10 (71)	2 (50)	4 (12)	2 (15)	1 (50)
FAST stage[4]	5	4 (14)	3 (21)	1 (25)	4 (12)	2 (15)	0 (0)
	6	19 (66)	8 (58)	1 (25)	21 (64)	10 (77)	1 (50)
	7	6 (20)	3 (21)	2 (50)	8 (24)	1 (8)	1 (50)
Withdrawal before 12 weeks[5]	No	24 (83)	11 (79)	3 (75)	23 (70)	10 (77)	1 (50)
	Yes	5 (17)	3 (21)	1 (25)	10 (30)	3 (23)	1 (50)
Gender[6]	Female	23 (79)	10 (71)	4 (100)	21 (64)	9 (69)	1 (50)
	Male	6 (21)	4 (29)	0 (0)	12 (36)	4 (31)	1 (50)
Delusions [7]	No	22 (79)	9 (64)	3 (75)	24 (73)	9 (69)	2 (100)
	Yes	6 (21)	5 (36)	1 (25)	9 (27)	4 (31)	0 (0)
Hallucinations [8]	No	24 (86)	10 (71)	4 (100)	21 (64)	12 (92)	0 (0)
	Yes	4 (14)	4 (29)	0 (0)	12 (36)	1 (8)	2 (100)

1. 12 week score missing from 4 cases, therefore final N=91; **2.** No memantine: $F(2,45)=1.6$, p=0.2; memantine: $F(2,44)=0.3$, p=0.7; **3.** No memantine: Fisher's exact p=0.2; memantine: Fisher's exact p=0.4; **4.** No memantine: Fisher's exact p=0.5; memantine: Fisher's exact p=0.6; **5.** No memantine: Fisher's exact p=1; memantine: Fisher's exact p=6; **6.** No memantine: Fisher's exact p=0.6; memantine: Fisher's exact p=1; **7.** Baseline score missing for one individual from the no memantine group, no memantine group based on n=46. No memantine: Fisher's exact p=0.7; memantine: Fisher's exact p=1; **8.** Baseline score missing for one individual from the no memantine group, no memantine group based on n=46. No memantine: Fisher's exact p=0.4; memantine: Fisher's exact p=0.01.

5.3.2 Primary analysis: *MAPT* haplotype and worsening psychotic symptoms

A subset of patients had MMSE scores recorded at both baseline and 12 weeks. Cognitive decline over 12 weeks was examined in cases from this subset with a baseline MMSE greater than 4 (chosen to avoid floor effects, n=38). The non-parametric Mann-Whitney U test showed no evidence of a relationship between change in MMSE score over the 12 week period and the *MAPT* haplotype (z=-0.1, p=0.9). Similarly there was no evidence of an effect of memantine on change in MMSE score (z=0.2, p=0.8).

Four patients (all from the MAIN-AD cohort) did not have complete 12 week data for neuropsychiatric symptoms, and these were there excluded, leaving 91 patients. Participants with a baseline NPI score of 12 were removed from the analysis to avoid floor effects: three participants had the maximum delusions score and one had the maximum hallucination score. Therefore 88 cases were included in the delusions analysis and 90 in the hallucinations analysis.

The primary analysis used logistic regression to evaluate the effect of *MAPT* haplotype and memantine treatment on worsening delusions and hallucinations.

Firstly, the demographic and clinical variables along with memantine treatment and *MAPT* haplotype were assessed in univariate analyses. The resulting odds ratios for worsening delusions and hallucinations are shown in Table 5-2 and Table 5-3 respectively.

With respect to delusions, there was no main effect of either memantine or *MAPT* haplotype and the worsening of this symptom. However in allelic analysis the H2 allele (shown in the lower section of Table 5-2 was associated with a 1.7 fold elevated risk for worsening of delusions (95% CI: 1.03-2.7, p=0.04).

No other clinical or demographic variables were associated with worsening delusions.

Table 5-2 Logistic regression: change in delusions (N=88)

Risk Factor		Delusions [Mean ±SD / Frequency (%)]		Odd ratio (95% CI)	p-value
		No change/better (n=78)	Worse (n=10)		
Age		83 (7.7)	80 (9.2)	0.96 (0.9-1.03)	0.3
Gender	Female	56 (72)	7 (70)	Reference	-
	Male	22 (28)	3 (30)	1.1 (0.3-4.6)	0.9
FAST stage	5	13 (17)	1 (10)	Reference	-
	6	46 (59)	7 (70)	2 (0.2-17.6)	0.5
	7	19 (24)	2 (20)	1.4 (0.1-16.7)	0.8
Delusions baseline	No	60 (77)	7 (70)	Reference	-
	Yes	18 (23)	3 (30)	1.4 (0.3-6.1)	0.6
Antipsychotic	No	42 (54)	7 (70)	Reference	
	Yes	36 (46)	3 (30)	0.5 (0.1-2.1)	0.3
Memantine	No	38 (49)	4 (40)	Reference	-
	Yes	40 (51)	6 (60)	1.4 (0.4-5.4)	0.6
MAPT	H1/H1	52 (67)	4 (40)	Reference	-
	H2*	26 (33)	6 (60)	3 (0.8-11.6)	0.1
MAPT allele	H1	126 (81)	12 (60)	Reference	
	H2	30 (19)	8 (40)	1.7 (1.03-2.7)	0.04

For hallucinations, there was also no main effect of memantine however the H2* group was associated with worsening hallucinations after 12 weeks (OR: 4.8, 95% CI: 1.2-20.3, p=0.03). Carriage of the H2 allele (see lower section of Table 5-3) was associated with a 1.9 fold increased risk of worsening hallucinations over the 12 week period (95% CI: 1.2-3.1, p=0.01).

No other clinical or demographic variables were associated with worsening hallucinations.

Table 5-3 Logistic regression: change in hallucinations (N=90)

Risk Factor		Hallucinations [Mean ±SD / Frequency (%)]		Odd ratio (95% CI)	p-value
		No change/better (n=80)	Worse (n=10)		
Age		83 (7.2)	79 (11.8)	0.9 (0.9-1.02)	0.1
Gender	Female	57 (70)	8 (80)	Reference	-
	Male	24 (30)	2 (20)	0.6 (0.1-3)	0.5
FAST stage	5	11 (14)	3 (30)	Reference	-
	6	49 (61)	6 (60)	0.5 (0.1-2.1)	0.3
	7	20 (25)	1 (10)	0.2 (0.02-2)	0.2
Hallucinations baseline	No	60 (74)	8 (80)	Reference	-
	Yes	21 (26)	2 (20)	0.8 (0.2-3.8)	0.7
Antipsychotic	No	40 (50)	10 (100)	Reference	-
	Yes	40 (50)	0 (0)	-[1]	-
Memantine	No	41 (51)	3 (30)	Reference	-
	Yes	39 (49)	7 (70)	2.5 (0.6-10.2)	0.2
MAPT	H1/H1	54 (68)	3 (30)	Reference	-
	H2*	26 (32)	7 (70)	4.8 (1.2-20.3)	0.03
MAPT allele	H1	130 (81)	11 (55)	Reference	
	H2	30 (19)	9 (45)	1.9 (1.2-3.1)	0.01

[1]OR generation not possible due to zero cases worsening among those taking antipsychotics

5.3.3 Secondary analysis: stratification by memantine treatment

The second part of the analysis aimed to assess the interaction between *MAPT* haplotype and memantine treatment. The sample was first stratified by memantine treatment and the effect of the *MAPT* haplotype was examined in each stratum. The risk associated with the H2* haplotype and worsening hallucinations was still present in the memantine group (OR: 7.3, 95% CI: 1.2-43.4, p=0.03) and not present in the placebo group. Similarly, worsening delusions was significantly associated with the H2* group (OR: 15. 95% CI: 1.56-144.2, p=0.02), this effect was not observed in the placebo group. The memantine by *MAPT* interaction was then tested in the final regression model. For hallucinations there was no significant interaction between the

memantine and the *MAPT* haplotype, whereas for delusions a significant interaction was observed (p=0.04). Although significant, the very wide confidence intervals and inspection of the regression diagnostics for this final model indicate that neither are a good fit for the data. The best models generated from this analysis are therefore those that do not include the interaction term, i.e. the stratified analyses (Table 5-4 and Table 5-5).

Table 5-4 Logistic regression: change in hallucinations stratified by treatment arm (N=90)

Risk Factor		No memantine (n=44) [Frequency (%)]				Memantine (n=46) [Frequency (%)]			
		Hallucinations		Odds ratio (95% CI)	p-value	Hallucinations		Odds ratio (95% CI)	p-value
		No change/better (n=41)	Worse (n=3)			No change/better (n=39)	Worse (n=7)		
MAPT	H1/H1	25 (61)	1 (33)	Reference	-	29 (74)	2 (73)	Reference	-
	H2*	16 (39)	2 (66)	3.1 (0.26-37.36)	0.37	10 (26)	5 (27)	7.3 (1.21-43.44)	0.03

P-value for interaction=0.58

Table 5-5 Logistic regression: change in delusions stratified by treatment arm (N=88)

Risk Factor		No Memantine (n=42) [Frequency (%)]				Memantine (n=46) [Frequency (%)]			
		Delusions		Odds ratio (95% CI)	p-value	Delusions		Odds ratio (95% CI)	p-value
		No change/better (n=38)	Worse (n=4)			No change/better (n=40)	Worse (n=6)		
MAPT	H1/H1	22 (58)	3 (75)	Reference	-	30 (75)	1 (17)	Reference	-
	H2*	16 (42)	1 (25)	0.46 (0.04-4.82)	0.51	10 (25)	5 (83)	15 (1.56-144.2)	0.02

P value for interaction =0.04

5.4 Summary of findings

5.4.1 Summary of primary analysis

1. 4.8-fold increased risk of worsening hallucinations over 12 weeks associated with individuals with at least one H2 allele.
2. 1.9-fold and 1.7-fold increased risk of hallucinations and delusions worsening respectively associated with the H2 allele.

5.4.2 Summary of secondary analysis

1. The association described above was still present in individuals taking memantine but not in the no memantine group, although stratification by treatment significantly reduced the sample size.

5.5 Discussion

These results are the first to implicate the H2 allele of the extended *MAPT* haplotype in the worsening of psychotic symptoms in dementia. Specifically carriers of at least one H2 allele (the H2* group) had a nearly 4.8-fold increased risk of worsening hallucinations over 12 weeks than H1 homozygotes, while there was no significant increased risk for delusions. Allelic analysis supported this conclusion with the H2 allele being associated with a 1.9-fold increased risk of worsening hallucinations and also a 1.7-fold increased risk of worsening delusions. This study brings an important dimension to research on tau and psychotic symptoms in AD. Previously, research has been limited to post-mortem studies, which can only draw conclusions about the extent of pathology at death, and genetic studies which do not examine progression of symptoms. Here it has been shown that the extended *MAPT* haplotype may cause worsening delusions and hallucinations in individuals matched for clinical disease severity, suggesting that the effect on psychotic symptoms of the tau pathology previously reported (Zubenko, Moossy et al. 1991, Forstl, Burns et al. 1994, Murray, Kirkwood et al. 2013) may not simply be mediated through greater disease severity.

It is important to highlight that there was no association between the *MAPT* haplotype and delusions or hallucinations at baseline. The evidence from this study would therefore suggest that the *MAPT* haplotype plays a disease modification role in AD such that H2 allele carriers have a greater risk of their psychotic symptoms worsening. This is supported by post-hoc analysis where there was no association with the *MAPT* haplotype and people who experienced psychotic symptoms at any stage of the 12 weeks (delusions: χ^2=1.7, df=1, p=0.2; hallucinations: χ^2=1, df=1, p=0.3). Twenty-four weeks of follow up data was available for the participants from the MAIN-AD study (n=58) and a further post-hoc analysis was conducted on this group under the same conditions as described for the 12 weeks analysis. The results supported the primary analysis in that the H2* group was significantly more likely to experience worsening delusions (χ^2=4.9, df=2, OR: 4.6, p=0.03), although in contrast to the 12 week analysis there was no association with hallucinations, perhaps indicating that longer term follow up periods are necessary to fully capture the course of psychotic symptoms.

This finding may have important clinical implications with respect to the treatment of psychotic symptoms in AD. The effective treatment of psychotic symptoms is an urgent unmet clinical need and there are significant challenges in addressing this while balancing the risks of unnecessary pharmacological treatment. The *MAPT* H2 allele may help in identifying people whose disease course is characterised by more enduring delusions and hallucinations who would therefore be most in need of close monitoring and treatment with pharmacological agents.

Given that more rapid cognitive decline is associated with psychosis and, more generally, cognitive decline is a central feature of AD, the *MAPT* haplotype was also examined in relation to this. The sample was restricted to 38 patients who had complete data for baseline and 12 week MMSE assessments and there was no evidence of a difference in rate of cognitive decline over this period between the haplotypes. Although a very small sample, this would suggest that the *MAPT* haplotype is not exerting its effect on the psychotic symptoms via more rapid cognitive decline, as is suggested previously in section 4.5 in relation to the *COMT* val158met polymorphism. The question of how the H2 allele would worsen psychotic symptoms when H1c is the putative risk allele for AD itself still remains however. This is a difficult question to answer based on the results here and also those that precede them pertaining to the functional significance of the *MAPT* haplotype and its role in AD generally.

With regard to the *MAPT* haplotype, in AD associated with Down's syndrome the H2 allele has been associated with an earlier disease onset (Jones, Margallo-Lana et al. 2008). In FTD that same association has been reported with the H2 allele along with the finding that it is also associated with glucose hypometabolism in the frontal cortex (Borroni, Yancopoulou et al. 2005, Laws, Perneczky et al. 2007).

Interestingly, PET studies in AD have identified frontal lobe hypometabolism as a correlate of delusional and, more broadly, psychotic symptoms (Sultzer, Brown et al. 2003, Sultzer, Mahler et al. 1995) while NFT burden in frontal areas has also been correlated with the same symptoms (Farber, Rubin et al. 2000, Zubenko, Moossy et al. 1991, Murray, Kirkwood et al. 2013). The underlying dysfunction associated with this PET neural correlate is not known but it will be important to establish whether the finding reported in FTD of frontal hypometabolism and the *MAPT* H2 haplotype is present in AD. This would help better explain the current findings although

a more complete explanation still could be arrived at from incorporating psychotic symptom data into the analysis as well.

The only other study to examine the *MAPT* gene in relation to psychosis in AD did not find an association (DeMichele-Sweet, Klei et al. 2011). It is important to highlight here that DeMichele-Sweet et al. examined the presence/absence of psychosis, whereas here the worsening of individual psychotic symptoms was analysed, these are two different types of analysis, the former being prevalence, the latter having a focus on disease course and how genetic variation may affect it. It is therefore possible that a different association would be found under each analysis even in a cohort of the same individuals, indeed there was no association between the *MAPT* haplotype and symptom status at baseline in this study. The DeMichele-Sweet et al. study was much larger than that reported here and in a well characterised cohort so it can be considered a robust negative with the respect to the phenotype investigated, but a second important distinction concerns just this. Specifically, the authors examined a psychosis phenotype (multiple/recurrent symptoms vs. no symptoms). However, in another subsequent study examining *APOE* in AD with psychosis, the *APOE**4 allele was found to be protective against hallucinations but not delusions in a dose dependent manner (Christie, Shofer et al. 2012). This finding raises several interesting points when considered alongside those reported in this study. The first is a justification for analysing delusions and hallucinations separately. There is strong indication that, for *APOE* at least, grouping psychotic symptoms as single/multiple recurrent symptoms or multiple one off symptoms may have masked associations in some of the large previous *APOE* studies (DeMichele-Sweet, Klei et al. 2011). It is not unreasonable to apply this logic to other genes, as was done here.

A second more general but noteworthy link between this study and that of Christie et al. is that the *4 allele was found to be protective against hallucinations while the H2 allele was found to be a risk factor for worsening symptoms in the present study. The *4 allele of *APOE* is the largest known genetic risk factor for sporadic AD itself and the H1 haplotype of *MAPT* has also been identified, although less consistently, as a risk factor for AD. It is therefore worth highlighting that the disease course of AD, specifically with respect to psychotic symptoms, may be negatively influenced by the very same alleles that are protective against AD.

The results presented here also include emerging evidence suggesting that memantine efficacy may be moderated by the *MAPT* haplotype. Inhibition and reversal of phosphorylated tau by memantine has been demonstrated in animal models and a reduction in CSF phospho-tau has been noted after one year of treatment in humans (Degerman Gunnarsson, Kilander et al. 2007, Li, Sengupta et al. 2004). This research is in itself in its very early stages and the mechanism by which memantine may exert this effect in humans is not known, therefore the current results should be considered as preliminary. In an analysis stratified by treatment, the worsening of delusions and hallucinations associated with the H2 haplotype persisted in the memantine group whereas no significantly increased risk of worsening was observed in the no memantine group, suggesting the H2 in combination with memantine may worsen delusions. There is precedent for psychotic symptoms worsening with memantine but it is only drawn from a limited number of case reports (Ridha, Josephs et al. 2005, Monastero, Camarda et al. 2007) and clinical trials have overwhelming found it to be a safe and well tolerated drug. In view of this and the limited understanding of memantine's mechanism of action, it would not be prudent to draw conclusions about this finding beyond stating that it may be an interesting avenue for future research and perhaps has the potential to shed some light on why memantine has been found to be ineffective in the treatment of neuropsychiatric symptoms in AD.

The most evident limitation of this study is the sample size. Even the largest RCTs are small by genetic standards but they do provide the optimum design for pharmacogenetic research and these two points are the principal limitation and strength of the pharmacogenetic component of this study. Ninety-five DNA samples were collected from a pool of 353 people who participated in the MAIN-AD and MAGD clinical trials. This is a reasonable number considering the frailty of this population of people (DNA collection commenced four years after the start of MAGD and three years after the start of MAIN-AD).

Measuring change using the NPI is not ideal, but in view of the parametric data assumptions being quite substantially violated there was no option but to create a new categorical outcome variable. This would have affected the statistical power of the study but grouping the participants into those who worsened and those who did not was considered to be the most clinically relevant grouping. There is of course a risk that an emerging symptom was not emerging at all but simply recurring and the time at which it first occurred was simply not during the follow up period of this

study. Similarly, a symptom which ameliorated may just represent the reverse. However, this is not a criticism unique to this study and is a product of investigating a psychiatric symptom which fluctuates with varying degrees of severity within individuals. There was only one mild dementia case in this sample, indicating that the vast majority of people were in the stage of dementia where psychotic symptoms are most likely to emerge. In AD, generally psychotic symptoms would occur frequently enough to be detected by the NPI (i.e. several times in a month), however it cannot be excluded that some episodes of delirium may have caused symptoms to be rated incorrectly as psychotic.

In summary, the evidence presented here is the first to indicate that the H2 allele of the extended *MAPT* haplotype negatively affects the course of delusions and hallucinations in AD. The next steps in this line of research should examine *MAPT* transcription in people with AD with and without psychotic symptoms in order to help understand the exact mechanisms underlying the results presented here. The results also suggest that genotyping for the H2 allele may enable the identification of a patient group with more enduring symptoms of psychosis and may contribute to the identification of individuals where pharmacological treatment may be necessary. Preliminary evidence also suggests that the *MAPT* haplotype may modify treatment response to memantine with important potential implications given the disappointing recent RCT evidence.

Chapter 6 Histamine H1 affinity of antipsychotics: relationship to mortality and a possible role of *HNMT* polymorphism

6.1 Summary introduction

Although there is a well established effect of long-term antipsychotic prescription on mortality in people with AD, the increased risk being around 1.5- to 1.8-fold (Schneider, Dagerman et al. 2005, Ballard, Hanney et al. 2009, Wang, Schneeweiss et al. 2005), the mechanisms causing this risk are not known. One working hypothesis is that the sedating effect of antipsychotics leads to secondary medical complications such as pneumonia and oedema which in turn lead to death (Ballard, Creese et al. 2010).

Not all antipsychotics are sedating and this is explained in part by their different affinities for the histamine H1 receptor. Blockade of H1 is the principal mechanism responsible for the sedative effect of antihistamines and the sedation observed in antipsychotics has been found to be closely related to their affinity for the H1 receptor.

Histamine is also of potential importance to mortality in dementia because of its role in the maintenance of fluid balance via vasopressin. Vasopressin induces water retention and is released during states of dehydration. Dehydration-induced vasopressin release is impaired in people with dementia and histamine action at H1 has been linked to vasopressin release (specifically, H1 antagonism suppresses vasopressin release in rats). Dehydration in people with dementia, particularly those in the moderate-severe stages, is common and a major challenge (Begum, Johnson 2010). The existing difficulties of maintaining hydration in people with dementia may be compounded by chronic blockade of H1 during long-term treatment with some antipsychotics and could therefore not only cause sedation but also further restrict the natural response to dehydration in people with dementia.

Although there are no polymorphisms in the H1 gene common enough in European samples, there is a non-synonymous SNP in the *HNMT* gene. *HNMT* is the enzyme responsible for the termination of histamine's action in the CNS. The less common T (ile) allele of the thr105ile polymorphism is associated with a decrease in enzyme activity, which is thought to lead to increased levels of histamine. Consistent with this, pharmacological inhibition of HNMT has been shown to increase histamine levels in animal studies.

Two experiments were therefore undertaken in order to examine the role of histamine in the mortality associated with antipsychotic treatment in AD and specifically the hypothesis that the histaminergic system plays an important role in mortality associated with antipsychotic drugs in AD. The first aimed to establish whether histamine H1 acting antipsychotics were associated with greater mortality than non-histamine H1 antipsychotics and the second investigated whether this relationship was modified by the *HNMT* thr105ile polymorphism.

6.1.1 Hypotheses

- High affinity histamine H1 antagonist antipsychotics will be associated with a greater risk of mortality over one year than those taking non-H1 antipsychotics and no antipsychotics at all.
- The HNMT polymorphism will modify mortality risk during exposure to H1 antipsychotics but not non-H1 antipsychotics.

6.2 Methods

6.2.1 Software

Excel: data cleaning, coding and organisation.

STATA: data analysis and coding.

6.2.2 Inclusion/exclusion criteria

Inclusion criteria:

- Probable, possible or definite Alzheimer's disease either clinically (according to the NINCDS-ADRDA criteria) or pathologically (according to CERAD/NIA-REAGAN criteria) confirmed.
- MMSE score available at first recorded antipsychotic visit.

Exclusion criteria:

- Baseline medication not recorded or recorded unknown.
- Baseline antipsychotic dose recorded as 'as required' (PRN).
- All medication not recorded or recorded unknown.
- Taking two antipsychotics of different classes at any stage during one year follow up.

6.2.3 Design and cohort

A total of 718 patients meeting the inclusion/exclusion criteria listed above were genotyped for the HNMT polymorphism (see section 2.6.1 for methods concerning genotyping) and analysed retrospectively. Patient data came from the following sources: ANM, ARUK, DCR, DEMVEST, Greek, Norway, OPTIMA.

In the event of the electronic databases lacking required information, the investigators responsible for each cohort were contacted.

6.2.3.1 Antipsychotic exposure and time

The antipsychotics prescribed to individuals were determined from individual databases and this list was cross-referenced with the MHRA website by a pharmacist (Dr Denise Taylor, University of Bath) who then classified each drug according to its H1 affinity (see Table 6-1).

Table 6-1 Antipsychotic classification according to H1 affinity and typical/atypical

Antipsychotic	H1 affinity	Typical/atypical
Chlorpromazine	High	Typical
Olanzapine	High	Atypical
Promazine	High	Typical
Quetiapine	High	Atypical
Thioridazine	High	Typical
Prochlorperazine	Medium	Typical
Flupentixol[1]	Low	Typical
Haloperidol	Low	Typical
Metoclopramide	Low	Typical
Risperidone	Low	Atypical
Sulpiride[1]	Low	Typical
Trifluoperazine[1]	Low	Typical
Zuclopenthixol	Low	Typical

[1]Little or no data available to classify drug

Antipsychotic exposure was coded with three levels:

0) no antipsychotic during follow up period (No AP)
1) Low or medium affinity histamine H1 receptor antagonist antipsychotic at some stage during follow up period (H1AP-)
2) High affinity antipsychotic during follow up (H1AP+).

Criteria for exposure classification:

- No AP: No record of any antipsychotic at any point during follow up.
- H1AP-: At least one record of a regular dose of antipsychotic.
- H1AP+: At least one record of a regular dose of antipsychotic.

Calculation of start date and time of follow up

The actual date of antipsychotic prescription was not available in any of the cohorts, therefore for those classified as prescribed antipsychotics the date of the first visit at which an antipsychotic was recorded was taken as the start date. The full decision process for calculating start date is shown in Figure 6-1. Dates of death were collected routinely as part of each cohort; any participant that left the study for any other reason was coded as censored. In the latter scenario the study exit date was taken as the last date of contact with the participant.

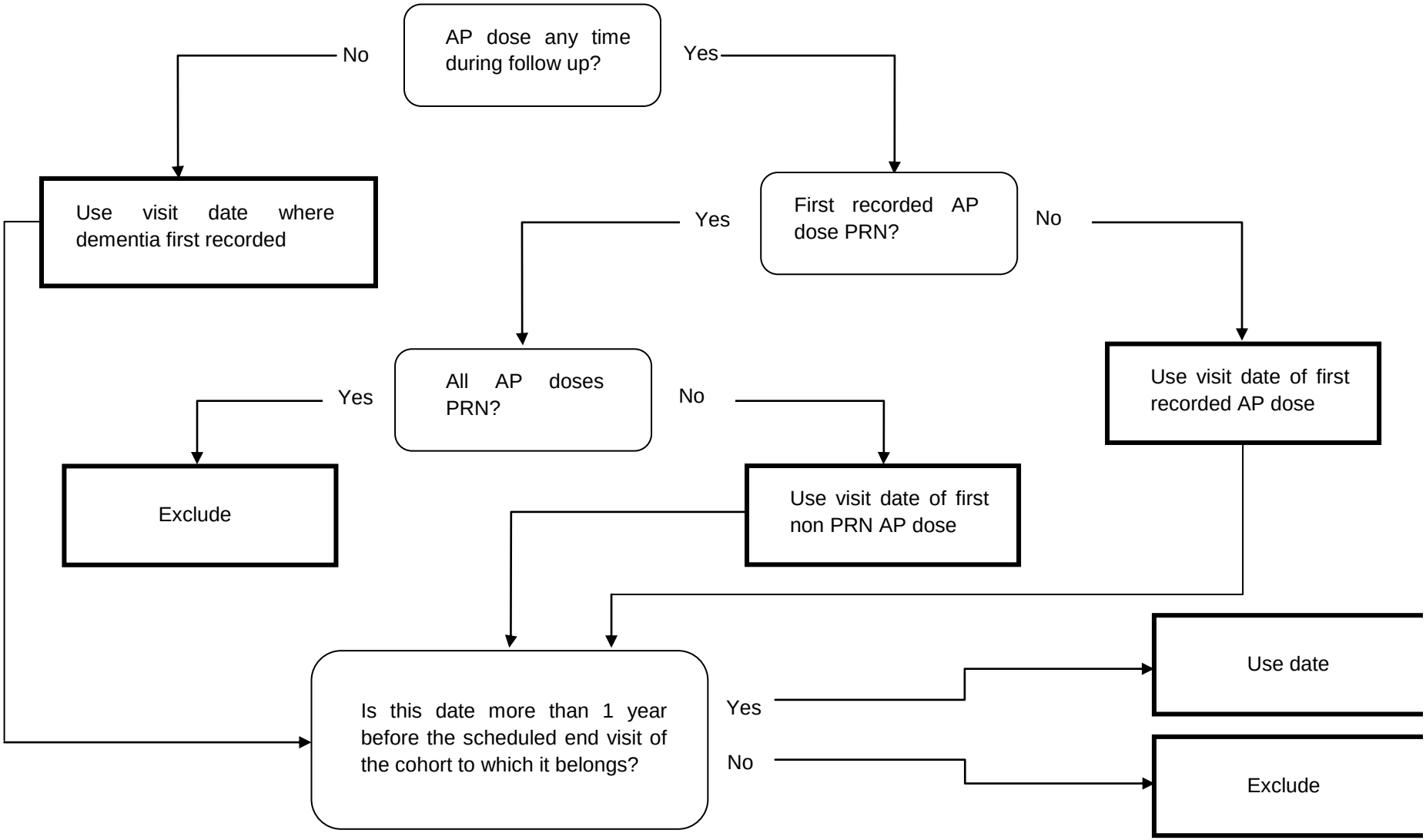

Figure 6-1 Decision process for estimating antipsychotic start date

6.2.4 Demographics and participant characteristics

Diagnosis, gender, age, level of cognitive impairment and concomitant medications were collected in all studies. Cognitive impairment was measured by MMSE while concomitant medication was recorded from patient records or by patient self-report (see section 2.3). The values of MMSE and age at recalculated start date were used for the analysis.

Missing MMSE scores were handled using the following criteria:

1) If MMSE score was available on both the prior visit and the visit after then the mean of those two scores was used to fill the missing value.
2) If the MMSE score had reached 2 or less (chosen because annual decline in AD is approximately 4 points on the MMSE and visits were spread a minimum of six months apart) in previous visits then zero was used to fill the missing value.
3) If three or more consecutive preceding scores were present then the average decline was taken from these three and subtracted from the score immediately before the blank to give the value to be used. The approach was also used for the reverse scenario. That is, the average decline for three or more subsequent scores was added to the score immediately after the blank.

Six and one blank MMSE scores were filled using criteria 2) and 3) respectively. No patients were suitable to be changed using criteria 1).

In instances where a clinical diagnosis of any dementia was present in life but the diagnosis changed on post mortem, the post mortem diagnosis was taken. Patients where dementia was not diagnosed in life but confirmed post mortem were not included in the analysis.

6.2.5 Statistical Evaluation

ANOVA, t-tests (or non-parametric equivalents) and chi square were used to compare means and frequencies of participant demographics respectively. Kaplan-Meier survival curve estimates were produced to assess mortality among the three drug groups and two *HNMT* groups (CC vs.

CT/TT) at one year. Following this, univariate Cox regression (with no AP and *HNMT* CC as the reference groups) was employed to generate and test the hazard ratios (HRs) of mortality associated with drug exposure and *HNMT* genotype. Antipsychotic mortality and it's modification by *HNMT* at 1 year was assessed in a Cox proportional hazards model with gender, age at first visit and MMSE at first visit included as covariates.

6.2.5.1 Power calculation

Assuming survival time as a quantitative trait, a MAF of 0.1 and a dominant genetic model (CC vs. CT/TT) this study would have >80% power to detect 2% variance in survival time while the same figures are arrived at for antipsychotic exposure, assuming 10% of people are taking antipsychotics. The interaction between *HNMT* genotype and antipsychotic exposure, assuming the same parameters just described, would have 75% power to detect 1% variance in survival and >80% to detect 2% variance.

6.3 Results

6.3.1 Descriptive statistics

Five hundred and sixty six (79%) participants were classified as not receiving an antipsychotic (no AP group) while thioridazine, risperidone and quetiapine were the most commonly prescribed antipsychotics (full details shown in Table 6-2). In total 60 patients were classified as receiving a non H1 acting antipsychotic (H1AP- group), while 102 were classified as receiving an H1 acting antipsychotic (H1AP+ group). A total of 12 patients were prescribed more than one antipsychotic during the one year follow up. Ten of these cases were receiving both a non-H1 acting antipsychotic and an H1 acting antipsychotic. As this study is concerned with identifying whether H1 blocking antipsychotics lead to increased mortality compared to non H1 blockers these 10 cases were excluded. The remaining two were receiving both olanzapine and quetiapine, so they were both classified as H1AP+, leaving 718 patients in the final analysis.

Table 6-2 Frequency of prescribed antipsychotics (N = 718)

Antipsychotic	**Frequency** (%)
None	566 (78.8)
Thioridazine	34 (4.7)
Chlorpromazine	14 (2)
Promazine	11 (1.5)
Sulpiride	5 (0.7)
Haloperidol	12 (1.7)
Zuclopenthixol	1 (0.1)
Trifluoperazine	2 (0.3)
Flupentixol	2 (0.3)
Olanzapine	15 (2.1)
Quetiapine	20 (2.8)
Risperidone	36 (5)

Of the 718 patients, 3, 24 and 4 were clinically diagnosed with vascular dementia (VaD), mixed AD/VaD, and DLB respectively (Table 6-3). However, at post-mortem all were found to either

have possible, probable or definite AD and so were included in the study (see table caption for further details).

Table 6-3 Dementia diagnosis frequency (N=718)

Clinical diagnosis	**Frequency** (%)
VaD	3 (0.4)[1]
Mixed AD and VaD	24 (3)[1]
Possible AD	26 (4)[2]
Probable AD	661 (92)[2]
Dementia with Lewy bodies	4 (0.6)[1]

[1]All pathologically confirmed as possible (n=3), probable (n=5) or definite (n=23) AD. [2]Pathological diagnosis available for 119 cases clinically diagnosed with possible or probable AD. Of these 7, 17 and 95 were pathologically diagnosed with, possible, probable and definite AD respectively.

Demographics by *HNMT* genotype are summarised in Table 6-4. CC genotypes were significantly more common among women (chi-square: χ^2 =5.5, df=1, p=0.02) and were associated with 1.7 point lower MMSE score than CT/TT genotypes (16.7 and 15 respectively, Mann-Whitney U: z=-2, p=0.03).

Table 6-4 Demographic characteristics of participants by HNMT genotypes (N = 718)

		***HNMT* Genotype** [Mean ±SD / Frequency (%)]	
		CC (n=564)	**CT/TT (n=154)**
MMSE		15 ± 8.7	16.7 ± 7.9[1]
Age		78 ± 7.6	78 ± 7.9
Gender	Female	383 (68)	89 (58)[2]
	Male	181 (32)	65 (42)
Dementia drug	No	342 (61)	86 (56)
	Yes	222 (39)	68 (44)
Antidepressant	No	465 (82)	123 (80)
	Yes	99 (18)	31 (20)
Sedative	No	505 (90)	140 (91)
	Yes	59 (10)	14 (9)
H1 antagonist	No	555 (98)	150 (97)
	Yes	9 (2)	4 (3)

[1]Mann-Whitney U: z=-2, p=0.03

[2]chi-square: χ^2 =5.5, df=1, p=0.02.

Demographics by antipsychotic exposure are summarised in Table 6-5. MMSE was significantly lower among those prescribed any type of antipsychotic, compared to those in the No AP group (Kruskal-Wallis: χ2=113.9, df=2, p<0.001). There were no differences in mean age or gender distribution between the three antipsychotic groups. With respect to concomitant medication, 43% of No AP cases were prescribed anti-dementia drugs (cholinesterase inhibitors or memantine), compared with 38% and 28% in the H1AP- and H1AP+ groups respectively (chi-square: χ^2=7.8, p=0.02). Both the prescription of sedatives and non-antipsychotic H1 antagonists were equally distributed across the three levels of antipsychotic exposure.

Table 6-5 Demographic characteristics of participants by antipsychotic status (N = 718)

		Antipsychotic Status [Mean±SD / Frequency (%)]		
		No AP (n=566)	**H1AP- (n=58)**	**H1AP+ (n=94)**
MMSE[1]		17.2 ± 8	8.8 ± 8.4	8.6 ± 6.7
Age		78 ± 7.5	78 ± 7.2	78 ± 8.5
Gender	Female	373 (66)	42 (72)	57 (61)
	Male	193 (34)	16 (28)	37 (39)
Dementia drug[2]	No	324 (57)	36 (62)	68 (72)
	Yes	242 (43)	22 (38)	26 (28)
Antidepressant	No	473 (84)	42 (72)	73 (78)
	Yes	93 (16)	16 (28)	21 (22)
Sedative	No	518 (92)	49 (84)	78 (83)
	Yes	48 (8)	9 (16)	16 (17)
H1 antagonist	No	557 (98)	58 (100)	90 (96)
	Yes	9 (2)	0 (0)	4 (4)

[1]Kruskal-Wallis: $\chi 2=113.9$, df=2, p<0.001
[2] chi-square: $\chi^2=7.7$, df=2, p=0.02

6.3.2 Primary analysis: antipsychotic mortality and *HNMT* genotype

The primary analysis consisted of univariate Cox regression to identify clinical and demographic risk factors for mortality. Significant predictors of mortality were then entered into a multivariate Cox regression model along with antipsychotic exposure and *HNMT* genotype to provide the final model. First, Kaplan-Meier survival curves were generated to illustrate mortality associated with different antipsychotics and *HNMT* genotypes. These illustrated a significant difference in survival estimates according to level of antipsychotic exposure (log-rank: $\chi^2=20.1$, df=2, p<0.001, Figure 6-2), while there was no difference in survival when *HNMT* genotypes were compared (**Error! Reference source not found.**). After generating the Kaplan-Meier curves it became apparent that study drop out date for 60 patients was the same as the start date, precluding them from entering into survival analysis, leaving 658 patients suitable for this part of the analysis.

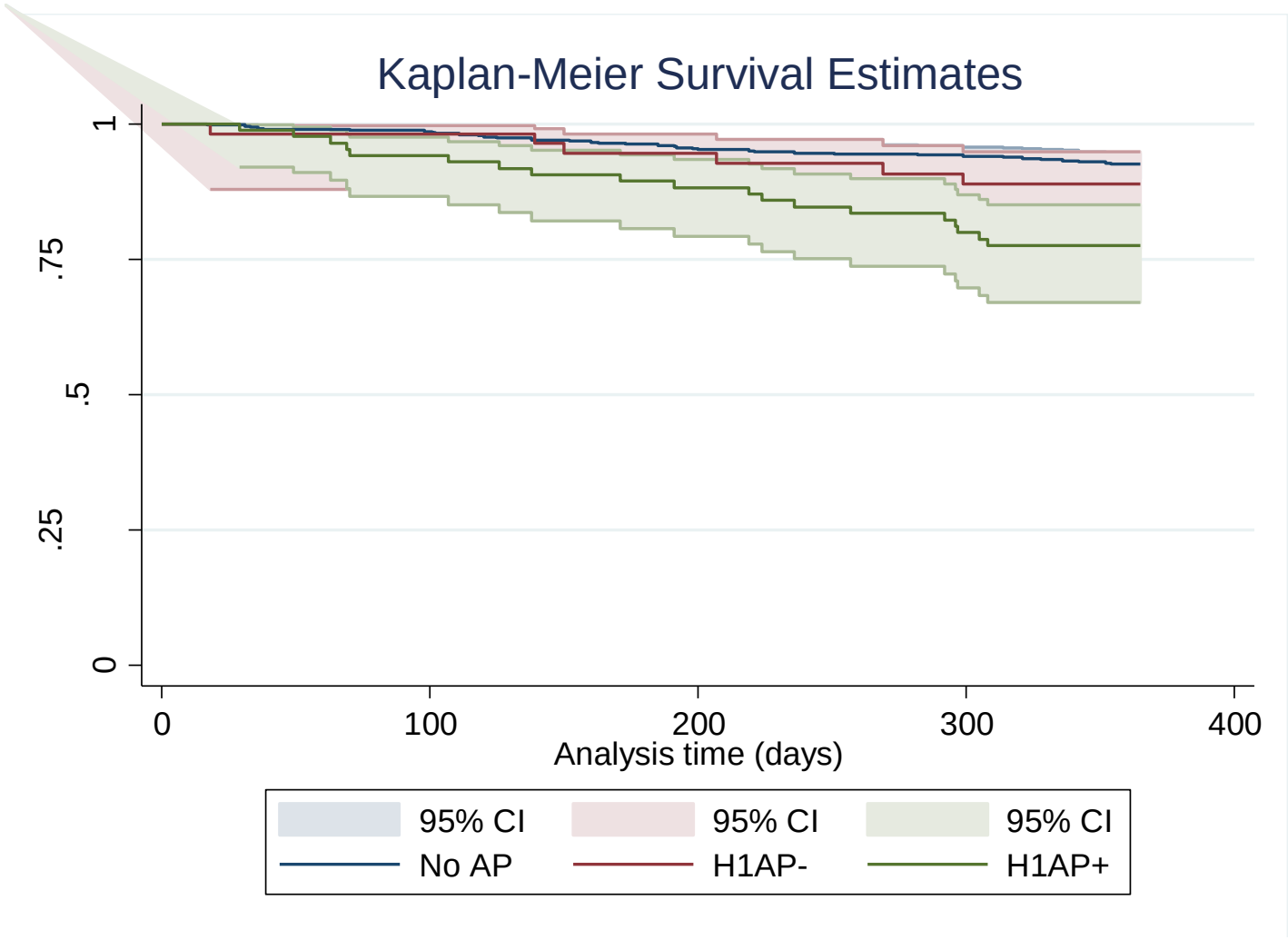

Figure 6-2 Kaplan-Meier curves for one year survival by level of antipsychotic exposure (Log-rank test: χ^2=20.1, df: 2, p<0.001)

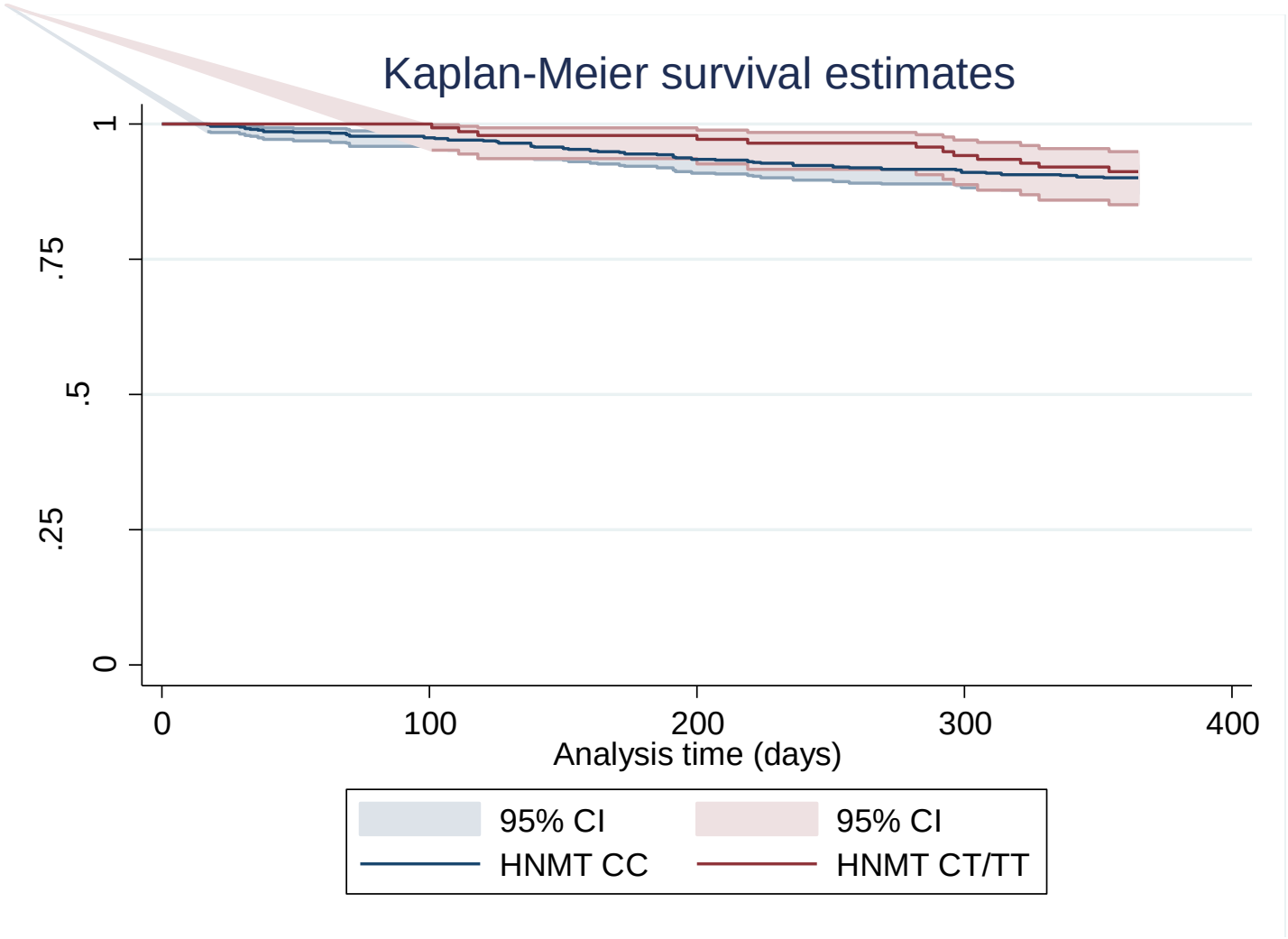

Figure 6-3 Kaplan-Meier curves for one year survival, comparing *HNMT* genotypes (Log-rank test: χ^2=0.3, df= 2, p=0.6):

The difference in antipsychotic survival shown in Figure 6-2 was supported by univariate Cox regression analysis yielding a hazard ratio (HR) of 3.3 ($p<0.001$) for mortality over one year in the H1AP+ group compared with the No AP group. Although an elevated risk for mortality was identified on the H1AP- group, it failed to reach significance (HR: 1.5, $p=0.4$). The full list of covariates examined is presented in Table 6-6. A lower MMSE and higher age were also strongly associated with mortality and anti-dementia drugs were found to be protective.

Table 6-6 Univariate analysis of risk factors for one year mortality (N=658)

		Mean±SD / Frequency (%)			
Risk factors		**Alive (n=596)**	**Dead (n=62)**	**Hazard Ratio (95% CI)**	**P-Value**
Age first visit		78 (7.5)	82 (7.7)	1.09 (1.05-1.13)	<0.001
MMSE first visit		16 (8.5)	8.7 (7.5)	0.91 (0.89-0.94)	<0.001
Gender	Female	395 (66)	37 (60)	Reference	-
	Male	201 (34)	25 (40)	1.3 (0.78-2.15)	0.3
Antipsychotic	No AP	479 (80)	37 (59)	Reference	-
	H1AP-	50 (8)	6 (10)	1.5 (0.65-3.62)	0.3
	H1AP+	67 (11)	19 (31)	3.3 (1.9-5.75)	<0.001
HNMT	CC	466 (78)	50 (81)	Reference	-
	CT/TT	130 (22)	12 (19)	0.84 (0.45-1.58)	0.6
Dementia drug	No	345 (58)	53 (85)	Reference	-
	Yes	251 (42)	9 (15)	0.25 (0.12-0.5)	<0.001

6.3.2.1 Multivariate analysis of antipsychotic mortality

The hazard ratio associated with antipsychotic exposure and *HNMT* genotype was adjusted for the covariates identified in the univariate analysis: age, gender, and MMSE.

There was a strong association between MMSE and anti-dementia drugs, with those exposed at baseline having significantly higher score than those not (median: 18 and 13 respectively, Mann-Whitney: $z=-7.3$, $p<0.001$). Due to the issue of colinearity between MMSE score and prescription of anti-dementia drugs, anti-dementia drug exposure was avoided as a covariate in the final model.

The multivariate Cox regression models for antipsychotic exposure and *HNMT* genotype are shown in Table 6-7 and Table 6-8 respectively. The association between the H1AP+ group and mortality persisted, albeit at a smaller effect size (HR: 1.9, $p=0.04$) in multivariate analysis adjusted for MMSE, age and gender. There was no significantly altered risk associated with the H1AP- group when compared with the no antipsychotic group (HR: 0.86, $p=0.7$). There was no association between the *HNMT* genotype and mortality.

Table 6-7 Multivariate analysis outcome of antipsychotic exposures for one year mortality (N = 658)

AP Status	Hazard Ratio (95% Confidence Interval)	P-Value
No AP	Reference	-
H1AP-	0.86 (0.35-2.1)	0.7
H1AP+	1.88 (1.05-3.4)	0.04

Adjusted for MMSE, age, gender

Table 6-8 Multivariate analysis outcome of *HNMT* genotypes for one year mortality (N = 658)

***HNMT* Genotype**	**Hazard Ratio (95% Confidence Interval)**	**P-Value**
CC	Reference	-
CT/TT	0.9 (0.5-1.8)	0.9

Adjusted for MMSE, age, gender

The antipsychotic exposed groups were then stratified by *HNMT* genotype to establish whether any effect modification was present. The results of this analysis are shown in Table 6-9. The effect of H1AP+ observed in the whole sample was still present at a similar effect size among the CC genotypes but not among the CT/TT genotypes (HR: 2.1, p=0.02; HR: 1.1, p=0.9 respectively). Among CC genotypes there was no significant increased risk associated with the H1AP- group (HR: 1.1, p=0.39). There was a zero mortality rate in the H1AP- group for CT/TT genotypes and as such it was not possible to estimate a meaningful hazard ratio and therefore not possible to suitably model the interaction between *HNMT* and antipsychotic exposure for this group. In order to estimate the model for this relationship one case from the H1AP- CC genotype group who died before one year was randomly allocated to the equivalent CT/TT genotype group and assigned the median survival time of 179 days. This was then repeated with two cases and finally three cases. Although the HR for the interaction did decrease with each additional allocation, in all cases it failed to reach significance (HR: 0.8, p=0.5; HR: 0.74, p=0.4; HR: 0.7, p=0.3 respectively).

Table 6-9 Multivariate analysis outcomes of antipsychotics exposure for one year mortality, stratified by HNMT genotypes (N = 658)

	HNMT CC (n=516)				HNMT CT/TT (n=142)			
	Alive (n=466)	Dead (n=50)			Alive (n=130)	Dead (n=12)		
Risk Factors	[Frequency (%)]		**Hazard ratio (95% CI)**	**P-value**	[Frequency (%)]		**Hazard ratio (95% CI)**	**P-value**
AP Status								
No AP	373 (80)	28 (56)	Reference	-	106 (82)	9 (75)	Reference	--
H1AP-	39 (8)	6 (12)	1.1 (0.43-2.7)	0.9	11 (8)	0 (0)	--[1]	--
H1AP+	54 (12)	16 (32)	2.1 (1.1-4.1)	0.02	13 (10)	3 (25)	1.3 (0.29-5.8)	0.7

Adjusted for MMSE, age, gender

P-value for the Interaction term=0.7

[1]Unable to estimate hazard ratio due to zero deaths in this group, see text

6.3.3 Secondary analyses

6.3.3.1 Analysis of mortality by anti dementia drugs

The association between anti-dementia drugs and reduced mortality in the context of antipsychotic exposure was then explored in more detail. Patients were classified as either exposed to an antipsychotic (of any type) or never exposed to an antipsychotic because of the very low cell numbers generated by stratification by anti-dementia drug. The dataset was stratified by anti-dementia drug exposure and Cox proportional hazard models were once again generated for each stratum. No significant increased risk was found in either those taking or not taking anti-dementia drugs (see Table 6-10).

Table 6-10 Multivariate analysis outcomes of antipsychotics exposure for one year mortality, stratified by anti-dementia drug exposure (N = 658)

Risk Factors	No anti-dementia drug (n=398)				Taking anti-dementia drug (n=260)			
	Alive (n=345)	Dead (n=53)			Alive (n=251)	Dead (n=9)		
	[Frequency (%)]		Hazard ratio (95% CI)	P-value	[Frequency (%)]		Hazard ratio (95% CI)	P-value
AP								
None	265 (77)	30 (57)	Reference	-	214 (85)	7 (78)	Reference	-
Yes	80 (23)	23 (43)	1.5 (0.81-2.7)	0.2	37 (15)	2 (22)	1.4 (0.3-8)	0.7

Adjusted for MMSE, age, gender and antidepressant exposure
P-value for the interaction term=0.6

6.4 Summary of findings

6.4.1 Summary of primary analysis

1. The H1AP+ group (histamine acting antipsychotics) carried a significantly greater risk of mortality over one year compared to the No AP group (HR: 1.9). There was no significantly increased risk associated with the H1AP- group.
2. The above association persisted in an analysis stratified by *HNMT* genotype: carriers of the CC genotype in the H1AP+ group had a 2.1-fold increased risk of mortality while there was no statistically significant increased risk among CT/TT genotypes.

6.4.2 Summary of secondary analysis

1. The effects of taking antipsychotics are not altered by anti-dementia drugs.

6.5 Discussion

In this study, patients taking high affinity H1 antipsychotic agents (H1AP+ group) had a significant 1.9 fold increased mortality risk compared with individuals not prescribed antipsychotics. Moreover, people taking non H1 blocking antipsychotics (H1AP-) had around the same mortality risk as the no antipsychotic group. This finding suggests that the histamine blocking action of antipsychotics may be a relevant mechanism in the already established mortality risk associated with their use. Further support for this hypothesis comes from the genetic component of this study. No increased mortality risk was found in the H1AP- group, across either of the *HNMT* groups, while the significant association with H1AP+ group persisted in CC genotypes, supporting the hypothesis that the histamine system may be important in mortality associated with antipsychotics in AD. It would be expected that relatively less sedation occurs with H1AP- antipsychotics and their lack of histaminergic action is the reason that no evidence of effect modification was observed by HNMT. Conclusions about the exact level of effect of H1AP- antipsychotics among CT/TT genotypes are hampered by the lack of deaths in this group, which made generation of HRs impossible. Excluding this group, collectively the HRs for each antipsychotic**HNMT* group were in the expected pattern. That is, H1AP- group carried the least risk (HR: 1.1), followed by the H1AP+ CT/TT genotypes (HR: 1.3) then finally by the H1AP+ CC genotypes (HR: 2.1). Although this is evidence of effect modification, as the interaction term was not significant it cannot be conclusively stated that this was a result of *HNMT* genotype.

Although the CC genotype is associated with greater HNMT enzyme activity which would lead to increased efficiency in the termination of action of histamine relative to CT and TT genotypes, the exact mechanism by which this may impact mortality among histamine acting antipsychotics is not known. Indeed, elucidating this is beyond the scope of a genetic association study and would need to be the subject of further molecular genetic work but what is already known about HNMT and histamine levels from *in vivo* studies is supportive of this hypothesis.

Pharmacological inhibition of HNMT increases CNS histamine levels (Duch, Bowers et al. 1978, Klein, Gertner 1981), and has been shown to induce waking and have non-sedative effects in animals when compared to placebo (Lin, Sakai et al. 1988, Tuomisto, Tacke 1986). The mechanism underlying increased mortality supported by the results presented here is therefore

one in which carriage of the higher activity CC genotype leads to decreased brain histamine levels, which in itself is not harmful in AD but along with long-term H1 blockade by antipsychotics in the H1AP+ group causes relatively higher levels of sedation due to less competition from endogenous histamine. Conversely, carriers of the *HNMT* CT/TT genotypes have relatively more endogenous histamine to compete with H1AP+ antipsychotics and thus have a greater level of H1 activation and therefore less sedation. Moreover, this mechanism may also impact mortality via dehydration. Long-term blockade of H1 receptors would reduce dehydration-induced vasopressin release, due to less histamine signalling, which is necessary to increase water retention. It is possible then that the relative depletion of histamine levels among *HNMT* CC genotypes would lead to a further relative decrease in histamine signalling and less histamine mediated vasopressin release. It is important to note that although dehydration is very common among individuals in long-term care facilities (Begum, Johnson 2010) it is not known how many people in the present sample would have been affected. It is therefore more appropriate to consider this as an additional risk mechanism, alongside sedation which, although again no measures of which were taken, would be much more predictably present among people in the H1AP+ group.

Post-hoc descriptive analysis was carried out to examine individual crude HRs associated with each antipsychotic. The table is shown in appendix A and as the frequencies of individual antipsychotics are very low in many cases it is difficult to draw firm conclusions. However there are several points that should be noted. Firstly, the very high HRs associated with chlorpromazine and thioridazine (7.1 and 5 respectively) suggest they may be driving much of the mortality among the H1AP+ group. Chlorpromazine is rarely used in current practice and thioridazine is no longer available; their presence in this sample is a result of a number of patients from older cohorts being included. This is a limitation of this study, they are both very high affinity H1 antagonists so this may be the main driver of the high mortality rate in these groups but it cannot be excluded that were they to be assigned to any arbitrary grouping of antipsychotics, that group would carry the increased mortality risk.

It is also noteworthy that every antipsychotic for which HRs could be generated showed a higher risk of mortality than risperidone. This does not support the hypothesis tested here as under this the H1AP+ antipsychotics would all be expected to carry greater risk than risperidone while the H1AP- antipsychotics would be expected to be carry a similar risk. It does however raise some

interesting questions for further study. Firstly, it could be that the hypothesis needs refining. As previously noted, typical antipsychotics carry an increased risk of mortality over atypicals, the mechanisms underpinning this are not known and the role of histamine has not been previously tested. The crude HRs, although not all statistically tested, suggest that perhaps the increased risk associated with typical antipsychotics is larger than that attributable to H1 antagonism. This does not necessarily mean that H1 antagonism is irrelevant, but perhaps means that splitting by typical/atypical then by histamine H1 action would be a more appropriate approach. This would certainly require a much larger sample and it is as such beyond the scope of the present study to statistically test this.

Quetiapine and risperidone are the two most commonly prescribed antipsychotics in people with dementia (Barnes, Banerjee et al. 2012). The crude HRs presented in appendix A indicate that quetiapine was associated with a higher mortality than risperidone. This is contrary to previous research which has in general shown quetiapine to have a lower mortality and adverse event rate than risperidone, probably due to the lower dosing of quetiapine relative to the recommended adult dose (Ballard, Waite 2006, Jin, Shih et al. 2013, Kales, Valenstein et al. 2007). Few antipsychotic mortality studies extend beyond 6 months and the one year difference in mortality observed here may represent a shift in the risk profile, although it should be acknowledged that this analysis was exploratory and the study was not powered to detect individual antipsychotic differences.

The genetic findings of this study and the classification of antipsychotics by their antihistamine action together provide some support for the hypothesis that sedation is a key event on the pathway from antipsychotic use to mortality, and these results suggest this line of work should be extended to other H1 acting psychotropic drugs commonly used in AD. This is covered in more detail in the General Discussion.

Although not directly related to the hypotheses of this study, a further result which warrants brief explanation is that taking anti-dementia drugs was strongly associated with reduced mortality. Those exposed to anti-dementia drugs at the start of the one year follow up had a 4-fold reduced risk of mortality compared to those who were not. This is perhaps an intuitive finding, but the literature surrounding mortality is mixed. At least two studies have reported that there is no

protection against mortality (Courtney, Farrell et al. 2004, Lopez, Becker et al. 2002), and it should be stated here that those taking antipsychotic medication were more likely to not be taking anti-dementia medication (particularly the H1AP+ group). It is therefore likely that this finding is in a large part the converse of the increased mortality associated with antipsychotics.

The strengths of this study include the sample size; this is the largest of its kind to date by some margin and provides a good dataset for further pharmacogenetic analysis. Moreover, all cases were well characterised in terms of concomitant medication and dementia severity. However, in any mortality study it would be preferable to have information regarding chronic diseases but the information available from the constituent cohorts of this sample was not consistent or in some cases reliable enough. It was the same case for cause of death. Information was known to be gathered from a number of different sources including death certificates, nurses' or family members' verbal reports. This was not considered reliable enough so only time to death was analysed in this study. There are known specific risk factors associated with antipsychotic use that could lead to death and a more sensitive analysis would be one that examines cause of death likely to be a result of these, e.g. stroke, pneumonia or cardiovascular related illness, although this would require an even larger sample size. Although this sample was large, and the overall number of people taking H1AP+ or H1AP- antipsychotics was comparable in size to other studies (n=94 and 58 respectively), once stratified by *HNMT* genotype the cell sizes did reduce significantly as a result of the T allele being relatively rare with a frequency of approximately 0.1. This would have significantly reduced the power of the genetic analysis, moreover it cannot be excluded that the different findings for CT/TT genotypes compared to CC genotypes was not a result of having 3 times as many individuals in the latter group and therefore much greater power to detect a difference. The final noteworthy limitation is the date used for start of antipsychotic prescription. This was an estimate and although participants were assessed at yearly, or more frequent, intervals it is still possible that the start date used could have been inaccurate by up to 12 months. In a cohort study such as this obtaining an exact start would be difficult but it would be preferable to have estimations of antipsychotic duration. This leads on to a general limitation of the study concerning being restricted by the designs of the cohorts from which the cases were drawn and will be returned to in the General Discussion.

Pharmacogenetic research in AD psychosis is important as it has the potential to yield information about the mechanisms of current treatments which is much needed in this area due to the paucity of pharmacological interventions currently available. Future research should seek to replicate this finding and extend research to the antipsychotic metabolising enzyme CYP2D6, which has been implicated in previous research (Pollock, Mulsant et al. 1995) but has not been investigated since.

The results presented here, gathered from classifying antipsychotics according to their H1 blocking action and supported by genetic data suggesting effect modification by *HNMT* genotype implicate the histaminergic system as an important mechanism in the mortality associated with these drugs. Specifically it was found that H1 blocking antipsychotics were the most harmful among carriers of the *HNMT* CC genotype with these individuals having a 2-fold increased mortality risk compared to those not taking antipsychotics, while there was no increased risk associated with non-H1 blocking antipsychotics. If replicated this may have clinically relevant implications for prescribing practice relating to other psychotropic H1 antagonists as well as confirming that sedation is indeed a key event in mortality associated with antipsychotic prescribing in AD.

Chapter 7 General discussion

The key aims of this thesis were to examine the role of common genetic polymorphisms in the prevalence, course and treatment of delusions and hallucinations in people with AD and DLB/PDD with a view to advancing current understanding of the mechanisms that underlie these symptoms and the responses associated with their treatment. Specifically, previous research and functional significance was used as a basis for examining the roles of the 5HTTLPR and *COMT* val158met polymorphisms in the prevalence of persistent delusions and hallucinations in some of the largest and best characterised cohorts of dementia to date. In addition, for the first time these two polymorphisms were evaluated with respect to rate of cognitive decline which is itself a key correlate of psychotic symptoms. The third approach used was to exploit the RCT methodology of two clinical trials in order to provide greater insight into the relationship between tau pathology and delusions and hallucinations by evaluating changes in these symptoms associated with the extended *MAPT* haplotype. Finally, the hypothesis that histamine H1 receptor antagonism is a key property of the well established mortality associated with antipsychotic use in AD was tested for the first time in a genetic association study.

7.1 Key themes

Four key themes have been identified from the preceding results chapters. They are discussed in detail below along with some broader interpretations.

7.1.1 The 5HTTLPR polymorphism has different effects on persistent delusions in AD and DLB/PDD

In this study the 5HTTLPR LL genotype has been found to be associated with a significantly higher risk for persistent delusions, but not hallucinations, in DLB/PDD relative to the SS genotype. This was in keeping with the hypothesis that was derived from research in AD. In contrast and contrary to the hypotheses there was no evidence of an association in AD. The limitations of these studies have already been discussed and although further study is required these results may indicate that different mechanisms, specifically genetically determined alterations in serotonin neurotransmission, underlie persistent delusions in AD and DLB/PDD. Global serotonergic changes are prominent in both DLB/PDD and AD (Halliday, McCann et al. 1992, Ohaea, Kondo et al. 1998, Chen, Eastwood et al. 2000). The study by Ohaea et al. (1998)

directly compared DLB/PDD patients and AD patients and found no difference in serotonin levels in either the frontal or temporal cortices between the two groups. There are few studies examining both AD and DLB/PDD but data from those that do and from analysis of individual diseases do not suggest that there are substantial differences in 5HTT sites between these dementias (Chen, Alder et al. 2002, D'Amato, Zweig et al. 1987). However dorsal raphe nucleus (DRN) 5HT neurons are affected in almost every patient with DLB/PDD while pathology in AD is comparatively limited in this region (Braak, Braak 1991, Francis, Perry 2007, McKeith, Dickson et al. 2005). It is therefore possible that the differential effect of the LL genotype found here is a reflection of the more severe involvement of brain stem 5HT synthesising nuclei in DLB/PDD than AD.

Other possibilities for this difference may include the greater amount of environmental factors that influence the presentation of hallucinations in AD such as impairments in visual acuity. From a neurobiological point of view, there are also much more widespread cholinergic deficits in DLB/PDD compared with AD (Perry, Haroutunian et al. 1994) which may be important given the substantial interaction between the cholinergic and serotonergic systems. 5HT plays both and inhibitory and excitatory role in ACh release via a number of different 5HT receptors, including $5HT_{1A}$ where it is excitatory (reviewed in (Lanctot, Herrmann et al. 2001). Differences in 5HT concentration resulting from the 5HTTLPR polymorphism may therefore impact the two dementias differently due to the differing extent of cholinergic deficits.

With respect to AD, tau pathology appears to be a key pathogenic correlate of psychotic symptoms, a hypothesis supported by the results presented here in Chapter 5. Given that tau pathology is found to a much lesser extent in DLB/PDD, this may be another key distinguishing mechanism which influences the effect of 5HTTLPR.

7.1.2 PFC dopamine levels may not underlie psychotic symptoms but may indirectly influence them via more rapid cognitive decline

The findings presented in this thesis do not suggest any involvement of the *COMT* val158met polymorphism in the aetiology of persistent psychotic symptoms in DLB/PDD or AD; this is in contrast to some but not all previous literature (Borroni, Grassi et al. 2006, Proitsi, Lupton et al.

2012, Sweet, Pollock et al. 2001, Borroni, Di Luca et al. 2006). However, a role for *COMT* val158met in the aetiology of psychotic symptoms in dementia cannot be ruled out. However, evidence that it appears to modulate D1 receptor density, rather than D2 receptors, and its better established role in cognitive performance, rather than psychosis, in people without dementia strongly suggests this line of work should continue to be extended in AD. This is also consistent with the evidence that DA blocking medications do not appear to alleviate psychotic symptoms in AD (Corbett, Smith et al. 2012). Moreover there is evidence contravening the hypothesis that levodopa alone induces hallucinations, most notably in one study high dose levodopa delivered intravenously failed to induce hallucinations in PD patients (Goetz, Pappert et al. 1998).

The fact that there was prior evidence of genetic association with both 5HTTLPR and *COMT* val158met was taken as a basis for further exploration of their possible roles in the aetiology of psychotic symptoms. The data from the cohort of people with AD was of a quality such that it was possible to examine the rate of cognitive decline associated with each polymorphism. This is important as there are plausible biological mechanisms to support a potential role of both 5HTTLPR and *COMT* val158met as mediators of cognitive impairment in people with AD. Consistent with the hypothesis, this study was able to demonstrate that the val/val genotype was associated with a 4-fold higher rate of annual cognitive decline than met/met carriers among patients with mild dementia. This is the first demonstration of this relationship in AD and potentially brings a new dimension to the current understanding of this polymorphism in the mechanisms underlying psychotic symptoms. Specifically, some of the discrepancies in prior studies may be explained by this relationship. More broadly, this finding may also have important implications for the enrichment of recruitment into clinical trials. People with mild dementia are frequently the target group for recruitment into clinical trials and this study highlights a possible subgroup which may decline at a faster rate and therefore generate more statistical power for randomised controlled trials (RCTs).

7.1.3 Evidence of genetic models of psychotic symptoms in dementia

Two principal hypotheses exist concerning the genetic epidemiology of psychotic symptoms in dementia. Firstly, in AD there is evidence (from heritability and post-mortem studies) to suggest that presence of persistent psychotic symptoms constitutes a distinct AD phenotype. Under this

model there are genes that predispose individuals to AD with psychosis (the heterogeneity model). Secondly there are genes that modify the course of AD to result in psychotic symptoms (Sweet, Nimgaonkar et al. 2003). This study found no support for 5HTTLPR or *COMT* val158met under either of these models in AD.

The finding that the *MAPT* haplotype is associated with a worse course of psychotic symptoms supports the disease modification model whereby in that the H2 allele was associated with a worsening of symptoms over 12 weeks (although full exploration of this would require the addition of a control group). Interestingly however it was the allele associated with a protective effect against AD itself, H2, which was associated with the worse outcome in this study. *MAPT* expression appears to be affected by the extended haplotype and it is proposed that the 4R isoform has a greater propensity to aggregate into NFTs and so increased expression by the H1c allele is a key driver of AD pathology. However not enough is known about the composition of NFTs in AD patients with psychotic symptoms compared to those without and it is therefore difficult to offer a mechanistic explanation as to why the H2 allele is associated with an increased risk. This will be an important area for future work. This research has also highlighted the value of examining the course of psychotic symptoms in AD and other dementias. Understanding risk factors for psychotic symptoms is important but it is also important to elucidate the finer detail of whether genes can affect the course of AD itself such that psychotic symptoms emerge more quickly or have a tendency to worsen. Specifically the results here point towards a genetic marker that may identify individuals in which pharmacological treatment may be indicated.

Although there has been no research into the existence of a psychotic sub-phenotype in DLB/PDD, the influence of the two polymorphisms under the two models outlined above was tested. Here, there was only support for 5HTTLPR in a disease modification capacity, specifically indicating the LL genotype becomes a risk for delusions once the neurodegenerative process has set in.

Finally, the results described in section 7.1.2 above concerning the *COMT* polymorphism and rate of cognitive decline suggest that perhaps the model of genetic influence on psychotic symptoms in AD should be expanded to include disease modifying genes that may indirectly affect psychotic symptoms via their influence on other clinical correlates.

7.1.4 Histaminergic neurotransmission is an important mechanism of mortality associated with antipsychotic treatment

The results from Chapter 6 demonstrated that high affinity antihistaminergic antipsychotics carry a greater mortality risk. Specifically, the high affinity antihistamine (H1AP+) antipsychotics carried a 1.9 fold increased risk of mortality over one year when compared with people not taking antipsychotics, while those in the lower affinity (H1AP-) group had approximately the same mortality risk as those not taking antipsychotics. Moreover, when stratified by *HNMT* polymorphism the risk persisted among carriers of the high activity CC genotype only, while there was no statistically increased risk associated with the low activity group (CT/TT). Both of these lines of evidence support the hypothesis that histamine neurotransmission is an important pathway in the mortality associated with antipsychotic prescription. Until now this question had not been empirically addressed, although high affinity H1 antagonist antipsychotics were already known to be sedative. This evidence particularly justifies the removal of thioridazine and chlorpromazine from clinical practice in people with dementia and as they were among the highest affinity H1 antagonists with the highest individual mortality risks of the agents investigated also suggests a possible, though not a sole, mechanism for their harmful effects.

At this stage the genetic evidence is not strong enough to warrant the screening of patients to predict who will be susceptible to increased mortality. This is not least because it is likely that other pharmacological properties of antipsychotics are also likely to cause side effects associated with mortality and therefore histamine action is likely to explain just a portion of the variance. What is more immediately applicable, and itself worthy of further investigation, is that histamine H1 blockade in itself may be a harmful effect of pharmacological treatment in AD. Many drugs besides antipsychotics are potent antagonists of H1, perhaps most notable of these are some of the non selective serotonin reuptake inhibitor (non-SSRI) antidepressants which are commonly used in dementia, like trazodone and mirtazapine. Recently antidepressants in general were found to be significantly more common among people already prescribed antipsychotics (Barnes, Banerjee et al. 2012). Specifically, the proportion of people prescribed trazodone among the antipsychotic group was twice that of the no antipsychotic group. There were no specific figures

for mirtazapine but along with SSRIs, tri-cyclic antidepressants (of which mirtazapine is one) are commonly used types of antidepressants. A recent RCT showed mirtazapine to be associated with significantly more adverse reactions (ARs), though not deaths, than placebo. Of particular significance to the issues discussed here, the most common ARs were drowsiness and sedation (Banerjee, Hellier et al. 2011). It will be important to monitor the prescribing patterns of other psychotropic drugs as antipsychotic use continues to fall. The results presented here implicate histamine in the mortality associated with antipsychotics and a wider application would be to extend this hypothesis to other commonly used psychotropic drugs which block the H1 receptor. If it can be established that long-term blockade of H1 is indeed a common harmful mechanism of psychotropic drugs in AD then this information could be used to guide prescribing practice. Finally, with typical antipsychotics becoming increasingly uncommon and so making the traditional typical/atypical distinction redundant in dementia, these results highlight the possibility that classification according to H1 receptor affinity may be a useful system to identify the highest risk agents.

7.2 Strengths and limitations

7.2.1 The cohorts

All of the experiments conducted in this project were based on data from participants recruited into other projects. That is to say that some limitations were an unavoidable consequence of the limitations and diversity of the original studies. These limitations are discussed in detail in each of the results chapters however it should be stated here that the cohorts were all extremely well characterised, particularly relative to other studies in this area and this is one of the main strengths of the work presented in the preceding results chapters. In particular in the AD studies it was possible to control for exposure to antipsychotics which is of relevance given their use has been associated with lower serotonin concentration in AD (Chen, Alder et al. 2002). Longitudinal data were available for the majority of patients and were of sufficient quality to enable the application of strict inclusion criteria and still yield good sized samples. On this point, all of the samples were of a comparable or larger size than most of the previous literature.

Ideally patients with pathologically confirmed diagnoses would be used in all analyses; however there are simply not the numbers available in current UK brain banks with the quality of data required to fulfil the sample sizes necessary for these experiments. All patients were from sources using standardised clinical diagnoses and because patients were assessed longitudinally, revisions were made when appropriate which would have increased the confidence with which diagnoses were made. All AD patients were diagnosed in accordance with the NINCDS-ADRDA criteria (McKhann, Drachman et al. 1984). The NINCDS-ADRDA criteria yield 83% and 84% sensitivity and specificity respectively (Blacker, Albert et al. 1994), meaning that 83% of AD patients and 84% of people without AD are correctly identified. The figures for the consensus criteria for probable DLB are 83% sensitivity and 95% specificity (McKeith, Ballard et al. 2000). Sensitivity and specificity for a diagnosis of PD are similarly high at 91% and 98% respectively (Hughes, Daniel et al. 2002). The diagnosis of dementia in PD was made according to the DSM-IV criteria with the one year rule also being applied to distinguish from DLB. No clinical diagnosis can ever offer 100% specificity and sensitivity and it is a fact of current research that it is likely that some individuals with other pathology or even with an entirely different diagnosis will be included in clinically diagnosed research samples.

Current large scale brain donation programmes, such as the Brains for Dementia Research initiative underway in the UK, will lead to more longitudinally studied patients with pathologically confirmed diagnoses becoming available. This of course is a complicated project and it will be some time before pathologically confirmed samples with high quality clinical data of the sort demanded by psychiatric studies are numerous enough to be investigated in genetic studies.

7.2.2 Genetics

7.2.2.1 Ethnicity

Data on ethnicity was not available for the following cohorts: OPTIMA, MAIN-AD (for sites except London), Norway and DEMVEST. This does present issues with population stratification in genetic association studies and it is known that the frequencies of all the polymorphisms examined differ by ethnicity (the tau haplotype to the extent that H2 is not present at all in non-Europeans).

Examination of the 1000 genomes browser indicates some variation in genotype frequencies by ethnicity of the polymorphisms investigated here (http://www.ncbi.nlm.nih.gov/-variation/tools/1000genomes), particularly when comparing European and non-European populations and as highlighted previously in section 1.4.7.1 (Creese, Ballard et al. 2013) with respect to 5HTTLPR this can impact results.

Whilst it is appropriate to acknowledge this as a limitation, there was very low percentage of non-Europeans among the known ethnicities (2%) and there was no reason to suspect the frequencies of non-Europeans among the unknowns would be any different. All of the unknowns were from UK or Norwegian samples where the percentage of non-Europeans in the elderly populations is very low.

7.2.2.2 SNP selection

The genes in this project were not fully interrogated; only a single polymorphism in each was examined. Both *SLC6A4* and *COMT* contain variations at other loci which are known to further alter either transcriptional or enzyme activity (in the case of *SLC6A4* and *COMT* respectively), however these other variants mostly quite rare, occurring in less than 10% of individuals.

It is worth singling out the a/g SNP contained in the 5HTTLPR L allele only, rs25531, which has been shown to reduce L allele transcription to that of the S allele. As the triallelic 5HTTLPR/rs25531 locus can be grouped in two classes based on functional differences (L_A vs. S/L_G) this can be genotyped without compromised sample sizes (essentially around 5-10% of L alleles would be moved to the S allele group). The results of the AD and psychotic symptoms experiment were a resolute negative and it is considered unlikely that a relatively uncommon SNP would have shifted the results to significance. This is perhaps more of an issue with the finding that persistent delusions are associated with the LL genotype in DLB/PDD. The presence of the rs25531 G allele in this cohort could conceivably remove the association of the LL genotype, although based on the Wendland et al. (2008) MAF of 0.06 it would only be expected that approximately six people would be affected in the DLB/PDD cohort (Wendland, Moya et al. 2008). Future work should certainly genotype rs25531, this would have been done in the present project

were it not for time constraints: as outlined in section 2.6.2.1.2 of the methods chapter there were problems encountered in using the Wendland et al. (2006) protocol for genotyping 5HTTLPR and rs25531. In future it would preferable to explore the use of a Taqman custom SNP genotyping assay for this polymorphism.

The issues discussed above allude to a wider point relating to the candidate gene approach, whereby single genes or polymorphisms within them are examined for relationships with disorders or traits. It is not only likely that other loci within the same gene will be associated with psychotic symptoms but also likely that a number of different genes will be. It is commonly accepted that complex traits of psychiatric disorders are likely to be mediated by rare genetic variation at many loci, each contributing a small risk. The studies presented in these thesis are only powered to detect relatively large risks (OR >1.3) in common polymorphisms. While it cannot be excluded that these polymorphisms exert a large risk, it must be acknowledged that some of the comparisons presented here (e.g. Table 3-5 Logistic regression: 5HTTLPR persistent delusions (N=97) and Table 6-9 Multivariate analysis outcomes of antipsychotics exposure for one year mortality, stratified by HNMT genotypes (N = 658)are underpowered and the findings should not be over interpreted. Larger sample sizes are the main barrier to overcoming this problem. Here, over 1,300 samples were screened for adequate symptom evaluation but less than 700 (around 600 AD and 100 DLB/PDD) met the stringent criteria for accurate diagnosis of psychosis. On the one hand it is important to have a well powered study but on the other, there is little case for conducting a well powered genetic study if the phenotype under investigation is not satisfactorily characterised in the sample.

The candidate gene approach remains a useful method for the investigation of the relationship between genetic variants and psychotic symptoms in AD, provided a hypothesis driven approach is used. In this project two criteria were used to identify the polymorphisms to be investigated: 1) evidence that the gene in which the polymorphic locus lies plays a role in the pathogenesis of the symptom or trait; and 2) functional consequences: only polymorphisms where there is evidence of functional significance, either by alteration of enzyme activity or gene expression regulation (e.g. splicing and transcription) were chosen. Moreover, only relatively common polymorphisms were examined in order to maximise the power of the experiments and evidence from prior association studies was also used to strengthen the case for selection where possible.

7.3 Future directions

In general, this thesis brings together well-characterised cohorts from several sources, making a good foundation for subsequent research. While many of the patients in each study were readily available from existing cohorts, the databases as used in the analyses were constructed specifically for this project. There is much work still to be done on the questions addressed in this thesis and the databases in their current form therefore represent a good opportunity to extend research along the same themes outlined here with relative ease. Moreover there are now clear criteria which can be used to add cases to the databases, potentially allowing for even larger sample sizes; the main difficultly in genetic epidemiological research.

Broadly, it would be of great benefit to examine variation across all of *COMT* and *SLC6A4*, particularly when it is known that variation at other loci have functional consequences. This would be the only way to fully characterise the role of each gene in the pathogenesis of psychosis and has the potential to greatly advance current understanding; a vital point given the current lack of effective treatments. The arguably more intractable side of the psychosis research presented here is the phenotype. Breaking down the psychosis syndrome into delusions and hallucinations makes fewer assumptions about the underlying mechanisms which may or may not be shared by each symptom. This was also the rationale for investigating the rate of cognitive decline in Chapter 4. The search for endophenotypes will be greatly aided by looking beyond the classic psychopathological features of psychosis to other clinical correlates. Cognitive decline is perhaps the most important of these and future work should continue to examine the complex relationship between psychotic symptoms, rate of cognitive decline and genetic variation in order to help better characterise psychosis in AD and other dementias.

The evidence surrounding the tau haplotype and worsening of psychotic symptoms is interesting in light of the literature suggesting tau pathology is associated with psychotic symptoms in AD. Extending the follow up time is the natural next step for this research. Because *MAPT* is a putative AD risk gene it would be of particular interest to examine the disease course from the early stages through to the late to establish whether the tau haplotype shortens the time to psychosis onset or whether it causes symptoms to worsen once they do. This would allow

exploration of how psychotic symptoms progress and exactly how they are related to the AD course as a whole.

With regard to pharmacogenetics, this is a greatly understudied area in psychosis in AD, largely due to the practicalities of gathering cohorts large enough. There has only been one well controlled study to date (Dombrovski, Mulsant et al. 2010) with a sample size of 92 (44 of whom were taking risperidone, the remainder were prescribed citalopram). Although there were two smaller cohort studies prior to this they were less well controlled and only examined treatment efficacy with respect to the amelioration of psychosis (Engelborghs, Holmes et al. 2004, Angelucci, Bernardini et al. 2009). The sample size of 658 in this study is therefore considerably larger and although accurate start dates for drugs were not available it nevertheless provides an excellent platform on which to build future research. Firstly, the results presented here suggest that the histamine hypothesis of antipsychotic mortality is worth exploring in more detail. Non-genetic research should move towards atypical antipsychotic drugs as they are still commonly prescribed, while the examination of other H1 acting psychotropic drugs used in dementia may be of interest. Larger samples will be needed to examine histamine pathway genes as there are very few with a minor allele frequency >0.1. With regard to other genes, of particular interest is cytochrome P450 (*CYP2D6*), responsible for metabolising the majority of antipsychotics. The gene's metabolic capability is genetically determined and a 5-fold increase in side effects (principally sedation and extrapyramidal symptoms) has been associated with poor metabolisers (identified through the amount of debrisoquine metabolite excreted after oral administration).

7.3.1 GWAS and emerging technologies in genetic epidemiology: a focus on their impact on research into psychotic symptoms in dementia

The candidate gene approach was used in this thesis and, as with any scientific study, independent confirmation of these findings is required before the conclusions presented here can be fully accepted. There is evidence that meta-analysis can go some way to address the concern of low power in genetic association studies and in order for this to be done in an effective way the characteristics of every sample presented here have been described in detail. It must be acknowledged however that there are newer, higher throughput methods for genotyping and investigating other types of genetic variation which will, if the field follows the same path as

schizophrenia, become part of dementia psychosis research in time. Such methods can only be positive additions to this field, which at present somewhat lags behind other psychiatric genetics research.

Perhaps an intermediate step between candidate gene studies, which examine just one polymorphism in a gene, and genome wide association (GWA) studies, which cover the whole genome, is to examine greater amounts of genetic variation within the gene of interest. This approach is covered in section 7.2.2.2 while the remainder of this section will focus on genome-wide techniques.

Being able to explain the role of a single gene fully is preferable to basing conclusions concerning the genetics of complex symptoms on the effect of a single polymorphism. The logic here is that capturing more genomic variation is a positive attribute to a study. This leads to the natural conclusion that an appropriately powered study with full genome coverage would be among the optimum approaches in genetic epidemiology. GWA studies in the AD and the wider psychiatric literature are now commonplace and the recent promising findings and very large cohorts used in many ways render the candidate gene approach obsolete in these fields (by way of comparison in 2011 the Schizophrenia Psychiatric Genome Wide Consortium (Ripke, Sanders et al. 2011) carried out a GWAS on 51,695 individuals) .

GWA studies, in which hundreds of thousands of SNPs covering the entire genome are genotyped, are now routinely applied to research into schizophrenia and depression. GWA is a hypothesis free approach, an attribute which is particularly attractive in the study of complex phenotypes such as psychosis in dementia. For example, although there is evidence to support the role of dopaminergic and serotonergic polymorphisms in the aetiology of these symptoms, disease causing pathways are likely to be far more complex and include completely unstudied mechanisms about which, by their nature, it is impossible to generate hypotheses. Looking to schizophrenia research, it is clear that the GWAS approach has advanced understanding of this disorder, having identified pathways involved in neuronal function and perhaps more unexpectedly the immune system (Aberg, Liu et al. 2013). Such developments are yet to translate into clinical practice but one of the most interesting and clinically significant findings to

emerge has come from the study of large sections (tens of thousands to millions of base pairs) of abnormally deleted or duplicated DNA which are detected by next generation sequencing (NGS).

So-called copy number variation (CNV) associated with schizophrenia has also been identified in other disorders which are considered distinct clinical entities and previously thought of as biologically separate as well (Schreiber, Dorschner et al. 2013). This is an excellent example of the role newer technologies can play in not just understanding the biology of psychiatric disorders but challenging existing clinical distinctions which may lead to new perspectives for subsequent research and management. With regard to the research presented in this thesis, a similar application of these newer techniques would be to elucidate whether there are shared pathways which underpin psychotic symptoms across the DLB/PDD/AD spectrum.

The use of genome-wide methods in dementia psychosis is limited to one study (Hollingworth, Sweet et al. 2012); it included 1,299 patients with psychosis and 735 without but is still relatively underpowered for this type of study, a fact acknowledged by the authors. The phenotype under investigation in the Hollingworth et al. (2012) study is another important limitation and one to which a good deal of resource should be directed in subsequent research. A cross sectional evaluation of psychosis was employed and given what is known about the course and complexity of psychotic symptoms in AD (see section 1.2), it is very likely that this either underestimated and/or misclassified psychosis in some individuals. Moreover, it is still debatable whether examining a combined psychosis phenotype (comprising delusions and/or hallucinations) is appropriate, a point discussed in full in previous sections of this thesis. The GWAS and NGS approaches have much to offer this field of research but there needs to be a focus on addressing methodological issues like those just described. Thus at present, the most significant impact that new and emerging genotyping techniques can have on dementia psychosis research is to provided an impetus to iron out concerns like these. There is limited value in committing considerable resource to run experiments in diseases/disorders in which the phenotype under investigation is flawed. There is an urgent need for greater use of longitudinal psychiatric evaluations using standardised, validated tools. The cohorts presented in this thesis go some way to addressing this problem and it will be by combining this sort of robust phenoptypic data with emerging genetic technologies that important new discoveries will be made.

7.4 Concluding remarks

Psychotic symptoms are difficult to study in people with dementia and there is an urgent need for research in this area to be carried out in longitudinally assessed and well characterised cohorts. The experiments presented in this thesis have addressed this problem in AD and DLB/PDD and presented robust results which make a positive contribution to this field. Consistent with neurochemical evidence from *in vivo* and post mortem studies no evidence was found to support a role of the *COMT* val158met polymorphism in the aetiology of psychotic symptoms in AD or DLB/PDD. The 5HTTLPR LL genotype was found to be a risk factor for persistent delusions in DLB/PDD but not in AD, suggesting that there are perhaps different mechanisms which underlie these symptoms across different dementias. New evidence that the *COMT* val/val genotype is associated with more rapid cognitive decline advances current understanding of the complex relationship between psychosis, cognitive decline and genetic variation and may help explain the discrepancies in previous studies examining psychotic symptoms. The *MAPT* H2 haplotype was found to modify the disease course of AD, being associated with a worsening of psychotic symptoms, complementing previous reports from post mortem studies and extending them by indicating there is a direct relationship between tau and psychotic symptoms. There was also preliminary evidence indicating that the H2 haplotype modifies the treatment response to memantine: a potentially important finding given the disappointing RCT evidence surrounding this drug. Further evidence into treatments for psychotic symptoms in AD concerned the use of antipsychotics. The findings here provide support for the hypothesis that histamine antagonism is an important harmful property of antipsychotic use in AD, with the potential to guide future treatment decisions.

Broadly, this thesis underscores the potential of genetic studies to improve clinical practice concerning the management of complex traits such as psychotic symptoms in dementia. Using a genetic association approach this thesis identified polymorphisms in genes which may help elucidate the complex mechanisms underpinning psychotic symptoms and their treatment and therefore help clinical decisions in future.

Appendix

A. DSM-IV criteria for dementia

1. The development of multiple cognitive deficits manifested by both
   a. memory impairment (impaired ability to learn new information or to recall previously learned information)
   b. one (or more) of the following cognitive disturbances:
      - aphasia (language disturbance)
      - apraxia (impaired ability to carry out motor activities despite intact motor function)
      - agnosia (failure to recognize or identify objects despite intact sensory function)
      - disturbance in executive functioning (i.e., planning, organizing, sequencing, abstracting)
2. The cognitive deficits in 1a and 1b cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning.

3. The cognitive deficits in Criteria A1 and A2 are not due to any of the following:
   a. other central nervous system conditions that cause progressive deficits in memory and cognition (e.g., cerebrovascular disease, Parkinson's disease, Huntington's disease, subdural hematoma, normal-pressure hydrocephalus, brain tumor)
   b. systemic conditions that are known to cause dementia (e.g., hypothyroidism, vitamin B_{12} or folic acid deficiency, niacin deficiency, hypercalcemia, neurosyphilis, HIV infection)
   c. substance-induced conditions
4. The deficits do not occur exclusively during the course of a delirium.
5. The disturbance is not better accounted for by another Axis I disorder (e.g., Major Depressive Disorder, Schizophrenia).

B. NINCDS-ADRDA criteria for clinical diagnosis of AD

1. The criteria for the clinical diagnosis of PROBABLE Alzheimer's disease include:
    - dementia established by clinical examination and documented by the Mini-Mental test, Blessed Dementia Scale, or some similar examination, and confirmed by neuropsychological tests;
    - deficits in two or more areas of cognition;
    - progressive worsening of memory and other cognitive functions;
    - no disturbance of consciousness and;
    - onset between ages 40 and 90, most often after age65; and absence of systemic disorders or other brain diseases.
2. The diagnosis of PROBABLE Alzheimer's disease is supported by:
    - progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia) and perception (agnosia);
    - impaired activities of daily living and altered patterns of behaviour;
    - family history of similar disorders, particularly if confirmed neuropathologically; and
    - laboratory results of:
        - normal lumbar puncture as evaluated by standardised techniques;
        - normal pattern or non-specific changes in EEG; such as increased slow wave activity and;
        - evidence of cerebral atrophy on CT with progression documented by serial observation.
3. Other clinical features consistent with the diagnosis of PROBABLE Alzheimer's disease, after exclusion of causes of dementia other than Alzheimer's disease, include:
    - plateaus in the course of progression of the illness;
    - associated symptoms of depression, insomnia; incontinence, delusions, illusions, hallucinations, catastrophic verbal, emotional or physical outbursts, sexual disorders and weight loss;
    - other neurological abnormalities in some patients, especially with more advanced disease and including motor signs such as increased muscle tone, myoclonus or gait disorder;
    - seizures in advanced age and;
    - CT normal for age.
4. Features that make the diagnosis of PROBABLE Alzheimer's disease uncertain or unlikely include:
    - sudden, apoplectic onset;
    - focal neurological findings such as hemiparesis, sensory loss, visual field deficits and incoordination early in the course of the illness and;
    - seizures or gait disturbances at the onset or very early in the course of the illness.
5. Clinical diagnosis of POSSIBLE Alzheimer's disease:
    - may be made on the basis of the dementia syndrome, in the absence of other neurological psychiatric , or systemic disorder sufficient to cause dementia, and in the presence of variation at onset, in the presentation, or in the clinical course;
    - may be made in the presence of second systemic or brain disorder sufficient to produce dementia, which is not considered to be the cause of the dementia and;
    - should be used in research studies when a single, gradually progressive severe cognitive deficit is identified in the absence of other identifiable cause.
6. Criteria for diagnosis of DEFINITE Alzheimer's disease are:
    - the clinical criteria for probable Alzheimer's disease and histopathological evidence obtained from a biopsy or autopsy
7. Classification of Alzheimer's disease for research purpose should specify features that may differentiate subtypes of the disorder, such as:
    - familial occurrence;
    - onset before age 65;
    - presence of trisomy-21 and;
    - coexistence of other relevant conditions such as Parkinson's disease.

C. Revised consensus criteria for clinical diagnosis of dementia with Lewy bodies

1. *Central feature* (essential for a diagnosis of possible or probable DLB):
   - dementia defined as progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function;
   - prominent or persistent memory impairment may not necessarily occur in the early stages but is usually evident with progression;
   - deficits of tests of attention, executive function and visuospatial ability may be especially prominent.
2. *Core features* (two core features are sufficient for a diagnosis of probable DLB, one for possible DLB):
   - fluctuating cognition with pronounced variations in attention and alertness;
   - recurrent visual hallucinations that are typically well formed and detailed;
   - spontaneous features of Parkinsonism.
3. *Suggestive features* (if one or more of these is present in the presence of one or more core features, a diagnosis of probable DLB can be made. In the absence of any core features, one or more suggestive features is sufficient for possible DLB. Probable DLB should not be diagnosed on the basis of suggestive features alone):
   - REM sleep behaviour disorder;
   - severe neuroleptic sensitivity;
   - low dopamine transporter uptake in basal ganglia demonstrated by SPECT or PET imaging.
4. *Supportive features* (commonly present but not proven to have diagnostic specificity):
   - repeated falls and syncope;
   - transient, unexplained loss of consciousness;
   - severe autonomic dysfunction, e.g. orthostatic hypotension, urinary incontinence;
   - hallucinations in other modalities;
   - systematised delusions;
   - depression;
   - relative preservation of medial temporal lobe structures on CT/MRI scan;
   - generalised low uptake on SPECT/PET perfusion scan with reduced occipital activity;
   - abnormal (low uptake) MIBG myocardial scintigraphy;
   - prominent slow wave activity on EEG with temporal lobe transient sharp waves.
5. A diagnosis of DLB in less likely:
   - in the presence of cerebrovascular disease evident as focal neurologic signs or on brain imaging;
   - in the presence of any other physical illness or brain disorder sufficient to account in part or in total for the clinical picture;
   - if Parkinsonism only appears for the first time at a stage of severe dementia.
6. Temporal sequence of symptoms:
   - DLB should be diagnosed when dementia occurs before or concurrently with Parkinsonism (it it is present). The term Parkinson's disease dementia (PDD) should be used to describe dementia that occurs in the context of well established Parkinson's disease. In a practice setting the term that is most appropriate to the clinical situation should be used and generic terms such as Lewy body (LB) disease are often helpful. In research studies in which distinction needs to be made between DLB and PDD, the existing one year rule between the onset of dementia and Parkinsonism continues to be recommended. Adoption of other time periods will simply confound data pooling or comparison between studies. In other research settings that may include clinicopathologic studies and clinical trials, both phenotypes may be considered collectively under categories such as LB disease or alpha-synucleinopathy.

D. UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria

Step 1: Diagnosis of Parkinsonian syndrome

- Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions);
- And at least one of the following:
    - muscular rigidity;
    - 4-6 Hz rest tremor;
    - postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction.

Step 2: Exclusion criteria for Parkinson's disease

- History of repeated strokes with stepwise progression of Parkinsonian features;
- History of repeated head injury;
- History of definite encephalitis;
- Oculogyric crises;
- Neuroleptic treatment at onset of symptoms;
- More than one affected relative;
- Sustained remission;
- Strictly unilateral features after 3 years;
- Supranuclear gaze palsy;
- Cerebellar signs;
- Early severe autonomic involvement;
- Early severe dementia with disturbances of memory, language, and praxis;
- Babinski sign;
- Presence of cerebral tumour or communicating hydrocephalus on CT scan;
- Negative response to large doses of levodopa (if malabsorption excluded);
- MPTP exposure.

Step 3: Supportive prospective criteria for Parkinson's disease
(three or more required for a diagnosis of definite Parkinson's disease)

- Unilateral onset;
- Rest tremor present;
- Progressive disorder;
- Persistent asymmetry affecting side of onset most;
- Excellent response to (70-100%) levodopa;
- Severe levodopa-induced chorea;
- Levodopa response for 5 years or more;
- Clinical course of 10 years or more.

E. Crude hazard ratios for individual antipsychotics relating to analysis presented in Chapter 6

H1 classification	Antipsychotic	N	Deaths	Hazard ratio (95% CI)
H1AP-	Risperidone	34	2 (6)	Reference
H1AP+	Thioridazine	34	9 (26)	5 (1.1-22.9)
H1AP+	Chlorpromazine	14	5 (36)	7.1 (1.4-36.5)
H1AP+	Promazine	11	3 (30)	4.8 (0.8-28.6)
H1AP+	Quetiapine	18	2 (11)	2.1 (0.3-14.8)
H1AP-	Haloperidol	12	2 (17)	2.9 (0.4-20.9)
H1AP-	Sulpiride	5	2 (40)	1.8 (1.5-76.9)

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