Genetics of Dementia with Lewy Bodies

L.J.M. Vergouw

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Genetica van dementie met Lewy bodies

Leonie Johanna Maria Vergouw

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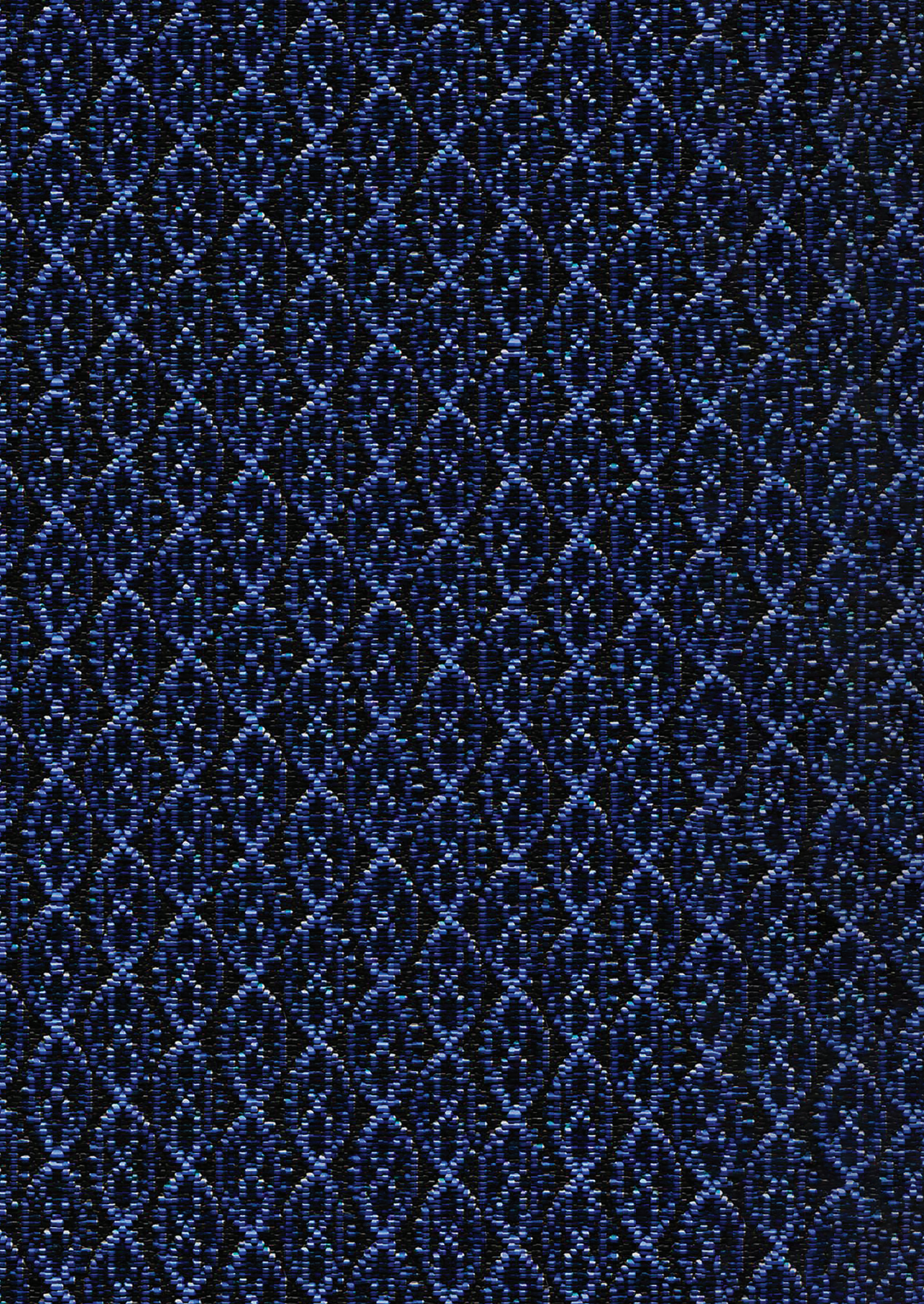

Part 1

General introduction

1.1

Chapter 1.1

Introduction to the thesis

Introduction to the thesis

Dementia with Lewy bodies (DLB) is a common neurodegenerative disease of the elderly. It accounts for approximately 5% of all patients with clinically diagnosed dementia.[1,2] However, the true prevalence is probably higher due to the fact that DLB is often overlooked and misdiagnosed.[3,4] Typical clinical features of DLB include progressive cognitive decline accompanied by parkinsonism, hallucinations, fluctuating cognition and REM-sleep behavior disorders. Other common symptoms are autonomic dysfunction, anxiety and depression.[5] Currently, there is no cure for DLB and treatment options are only available to lessen symptoms.[6] Patients with DLB have a median survival of approximately four years from diagnosis.[7] The mixture and severity of symptoms, lack of disease-modifying treatment options, and poor prognosis makes DLB a dreadful disease.

DLB shares clinical and pathological features with Parkinson's disease (PD) and Alzheimer's disease (AD).[6] Pathological hallmarks of DLB and PD are Lewy bodies and Lewy neurites.[8] This Lewy pathology is spread throughout the cortical regions of the brain in DLB, in contrast to PD where Lewy pathology is largely confined to subcortical regions, at least in the initial disease stages.[9] Additionally, AD pathology is observed in the majority of DLB patients.[10,11]

In contrast to genetic research in PD and AD, few genetic studies have been performed in DLB. Recently, a considerable genetic component has been suggested in the pathogenesis of DLB.[12] Nonetheless, only some genetic factors (the *apolipoprotein E* ε4 allele, and specific variants in the *glucocerebrosidase* and *α-synuclein* genes), previously associated with AD and PD, have also been associated with DLB.[13,14]

The aim of this thesis is to shed more light on the genetics of DLB, which could lead to a better understanding of the causes of DLB and its associated pathobiology. This knowledge could lead to the development of biomarkers, which are very important in the diagnostic and prognostic process, and may ultimately contribute to the identification of new targets for the development of disease-modifying treatments.

Chapter 1.2 provides an overview of the genetics of DLB. Little is known about possible differences between DLB patients with a positive family history of dementia or PD, as opposed to DLB patients with a negative family history of these diseases. In **Part 2** differences in phenotype between these two groups are described. Considering the overlap between DLB, AD and PD, in **Part 3** we investigated whether the known AD and PD genes are also associated with DLB. To increase the chances of finding genetic associations, we focused on two specific patient groups. We directed our analyses on DLB patients with a positive family history of dementia or PD in **Chapter 3.1** and on pathologically confirmed DLB patients with rapid disease progression (clinically suspected of Creutzfeldt-Jakob's disease) in **Chapter 3.2**. In **Part 4** and **Part 5**, the focus shifts to the search for novel genes associated with DLB. The *LRP10* gene was recently nominated as a novel gene associated with PD, PD dementia, and DLB[15], and is, therefore, the principal focus of **Part 4**. This gene was analyzed in clinically

diagnosed PD and DLB patients (**Chapter 4.1**), dementia patients with Lewy pathology, dementia patients with parkinsonism without Lewy pathology (**Chapter 4.2**), and patients with progressive supranuclear palsy (**Chapter 4.3**). In **Part 5**, a multimodal approach is used to search for novel genes. Cerebrospinal fluid (CSF) proteomic analysis in DLB patients is described in **Chapter 5.1**. This dataset was used in the **Appendix to Chapter 5.1** to combine genetic and proteomic data to find novel candidate genes in a pilot study. **Part 6** entails a general discussion of the thesis in the context of the current literature and provides suggestions for future research. Finally, **Part 7** summarizes the principal findings of the thesis.

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1.2

Chapter 1.2

An update on the genetics of dementia with Lewy bodies

Leonie J.M. Vergouw, Inger van Steenoven, Wilma D.J. van de Berg, Charlotte E. Teunissen, John C. van Swieten, Vincenzo Bonifati, Afina W. Lemstra, Frank Jan de Jong

Parkinsonism and Related Disorders 2017;43:1-8

Abstract

The genetic architecture of dementia with Lewy bodies (DLB) is increasingly taking shape. Initially, genetic research focused mainly on linkage and candidate gene studies in small series of DLB patients. More recently, association and exome sequencing studies in larger groups have been conducted, and have shown that several variants in *GBA* and the *APOE* ε4 allele are important genetic risk factors for DLB. However, genetic research in DLB is still in its infancy. So far, many genetic studies have been biased and performed in clinically and pathologically heterogeneous populations. Therefore, it is likely that multiple DLB-specific genetic determinants still have to be identified. To further our understanding of the role of genetics in DLB, future genetic studies should be unbiased and performed in large series of DLB patients, ideally with both a clinical diagnosis and pathological confirmation. The combination of genomic techniques with other research modalities, such as proteomic research, is a promising approach to identify novel genetic determinants. More knowledge about the genetics of DLB will increase our understanding of the pathophysiology of the disease and its relation with Parkinson's disease and Alzheimer's disease, and may eventually lead to the development of disease modifying treatments.

Introduction

Dementia with Lewy bodies (DLB) is a common neurodegenerative disease in the elderly.[1] DLB is characterized by progressive cognitive decline with variable combinations of fluctuating cognition, parkinsonism, visual hallucinations, neuroleptic sensitivity and rapid eye movement (REM)-sleep behavior disorders.[2] Clinical features of DLB are not specific to the disease and overlap with those of Parkinson's disease (PD) and Alzheimer's disease (AD).[1] In addition, neuropathological features also overlap between these diseases. Cortical Lewy bodies and neurites, which mainly comprise abnormal aggregated α-synuclein[3], are the pathological hallmarks of DLB, but are also observed in advanced PD and Parkinson's disease dementia (PDD)[4]. Furthermore, AD pathology is present in most DLB patients[4-6], which may aggravate the clinical manifestation of the disease and may increase the risk of mortality[7-9]. Due to these overlapping features, DLB is often considered as part of a spectrum with DLB placed between PD and AD (Figure 1).[10]

Over the last years, the genetic architecture of DLB is increasingly taking shape.[10-13] Defects in genes associated with PD (such as *α-synuclein* (*SNCA*)[14-17], *leucine-rich repeat kinase 2* (*LRRK2*)[18] and *glucocerebrosidase* (*GBA*)[19-21]) or AD (such as *presenilin 1* (*PSEN1*)[22-24], *presenilin 2* (*PSEN2*)[13,24,25], *amyloid precursor protein* (*APP*)[11,26], *apolipoprotein E* (*APOE*)[11,13,24,27-30] and *microtubule-associated protein tau* (*MAPT*)[31]) have also been associated with DLB. In addition to the clinical and pathological overlap, these findings also suggest a genetic overlap of DLB with PD and AD (Figure 1).[10]

In this article, we present a comprehensive overview of the genetics of DLB and discuss the genetic overlap of DLB with PD and AD. In addition, we describe promising genetic research methods, which in the near future will further our understanding about the pathophysiology of DLB and the clinicopathological spectrum between DLB, PD and AD.

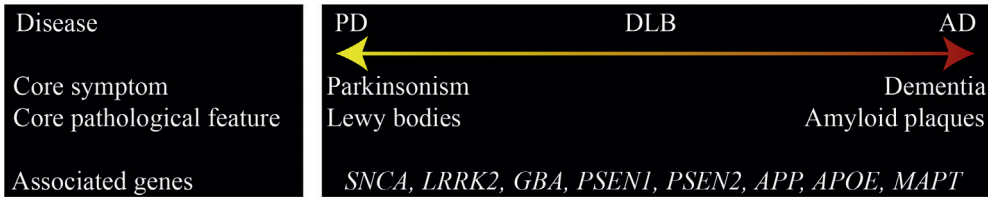

Figure 1: Disease spectrum of PD-DLB-AD (simplified representation).

Genetics of DLB

Multiple studies have been published on the genetics of DLB. The first genetic studies involving DLB patients mainly focused on families with multiple affected members with variable phenotypes ranging from DLB to PD and AD.[14-18,22-26,32-36] These studies used linkage analysis or a candidate gene approach to find rare variants (usually defined as variants with an allele frequency of less than 1% in the general population) with a large risk of disease development.[37,38] In such families, two disease-associated loci (2q35-q36 and 2p13[35-36]) and twelve disease-associated rare variants in six genes have been identified[11,13-18,22-26,32,34] (Table 1). Rare disease-associated variants have also been identified by candidate gene studies in series of unrelated DLB patients (Supplementary Table 1).[13,19-21,24,39-47] Some of these variants reside in genes previously associated with DLB in familial studies, which supports a role for these genes in DLB. Interestingly, rare disease-associated variants in *GBA* are often observed in unrelated DLB patients.[19-21,39-41,43]

Association studies in large cohorts of patients and controls generally take the approach of identifying common variants (usually defined as variants with an allele frequency of more than 1% in the general population) with a small to intermediate risk of disease development.[37,38] These studies with candidate genes have shown an association between DLB and, among others, the *APOE* ε4 allele[11,13,24,27-30] and the *MAPT* H1G haplotype.[31] A genome wide association (GWA) study, which is hypothesis free and typically identifies new disease-associated loci[37,38], has not yet been reported for DLB. Other relatively new and unbiased genetic approaches[37,38], such as whole exome and genome sequencing studies, have also not yet been reported.

Genes associated with DLB are discussed in more detail in the next sections.

Rare variants in SNCA

Several defects in *SNCA* (p.E46K, p.A53T variant and duplication) have been described in DLB patients with family members diagnosed with PD or PDD (Table 1).[14-17] A *SNCA* duplication was also found in a DLB patient without affected family members (Supplementary Table 1).[24] These defects were previously identified in multiple familial PD patients and are considered pathogenic.[14,16,48,49] Although evidence is scarce, there are some indications that specific genetic variability within *SNCA* could lead to different phenotypes in the PD-DLB spectrum. For example, the p.A30P variant[50] and duplications of *SNCA* are more often associated with PD and sometimes with PDD with a long disease course[51,52], whereas the p.E46K variant, the p.A53T variant and triplications are associated with PD and DLB with an early age of onset, severe clinical symptoms and a short survival[53]. The difference of phenotype with the type of variant may be related to the position of the variant and its impact on protein function.[14] Similarly, the kind of multiplication and genomic range of the *SNCA* multiplication may influence the clinical phenotype.[17]

Table 1: Rare disease-associated genetic variants in familial DLB.

Genetic characteristics				Clinical diagnosis	Family history				Pathological characteristics			References
Gene	**Protein**	**Chromosome location**	**Protein change**		**DLB**	**PD/PDD**	**AD or unspecified dementia**	**number of affected family members**	**Autopsy performed**	**Cortical Lewy pathology**	**AD-pathology**	
SNCA	α-synuclein	4q22.1	E46K	DLB**	no	yes	no	12	yes	yes	no	[14]
			A53T	DLB**	no	yes	no	3	yes	yes	no	[15]
				DLB	no	yes	no	4	no	NA	NA	[16]
			duplication*	DLB	no	yes	no	1	no	NA	NA	[17]
LRRK2	leucine-rich kinase 2	12q12	G2019S	DLB	no	yes	no	4	yes	yes	yes	[18]
PSEN1	presenilin 1	14q24.2	T440 deletion*	DLB**	no	yes	no	2	no	NA	NA	[22,23]
			A79V	DLB	no	no	yes	1	no	NA	NA	[24]
PSEN2	presenilin 2	1q42.13	A85V	DLB	yes	no	yes	5	yes	yes	yes	[25]
			R71W	DLB	no	no	yes	1	no	NA	NA	[24]
			D439A	DLB	no	yes	no	1	yes	yes	yes	[13]
APP	amyloid precursor protein	21q21.3	V717I	DLB/AD	UN	UN	UN	>1	yes	yes	yes	[11]
			duplication	DLB	no	no	yes	2	yes	yes	yes	[26]
SNCB	β-synuclein	5q35.2	P123H	DLB	yes	no	yes	7	yes	yes	yes	[32,34]

* Confirmed in a son with PD and dementia. ** Based on the clinical criteria by McKeith et al., 2005, no definite diagnosis was mentioned in article. DLB: dementia with Lewy bodies, PD: Parkinson's disease, PDD: Parkinson's disease dementia, AD: Alzheimer's disease, UN: unknown, NA: not available.

Rare variants in LRRK2

Mutations in *LRRK2* are an important genetic cause of PD. The most common mutation is p.G2019S, with mean frequencies ranging from 1% in sporadic, to 4% in familial PD worldwide.[54] However, the p.G2019S frequency varies significantly between populations, with higher frequencies in North African Arabs, Ashkenazi Jews and patients from the Middle East and southern Europe.[54,55] Disease-associated rare variants in *LRRK2* have rarely been found in DLB patients. Only one of 417 patients with clinical DLB and 355 patients with neuropathological confirmed Lewy body disease carried the p.G2019S mutation (Supplementary Table 1).[42] In addition, the p.G2019S mutation was only observed in one single patient with DLB from a family with several members affected with PD (Table 1).[18] This suggests that *LRRK2* disease-associated rare variants are, in contrast to PD, not a common cause of DLB.

Rare variants in PSEN1, PSEN2, APP

Mutations in *PSEN1*, *PSEN2* and *APP* are typically associated with familial AD, but have also been associated with other phenotypes including DLB.[56,57] Several defects (*PSEN1:* p.A79V variant and p.T440 deletion[22-24], *PSEN2:* p.R71W, p.A85V and p.D439A variant[13,24,25] and *APP:* p.V717I variant and duplication[11,26]) were found in families with DLB and dementia or PD (Table 1). Most of these defects (except from the *PSEN1* p.T440 deletion and *PSEN2* p.A85V variant) have previously been identified in (familial) AD and are considered pathogenic (except from *PSEN2:* p.R71W and p.D439A variant).[58-64]

There are a number of possible reasons for finding defects in *PSEN1, PSEN2* and *APP* in DLB patients. First, patients may have been misdiagnosed as having DLB instead of AD, as neuropathological confirmation was not always available. Second, in addition to AD pathology, Lewy pathology is frequently observed in patients with familial AD. Lewy pathology is found (especially in the amygdala) in more than 60% of the familial AD cases, and approximately 30% of *PSEN1* or *PSEN2* mutation carriers have cortical Lewy pathology.[65,66] Previous studies have suggested that specific genetic defects in *PSEN1* and *PSEN2* influence the amount of coincidental Lewy pathology in AD patients, which may lead to a more DLB-like phenotype in patients with a higher Lewy pathology load.[65,67,68]

Rare (and common) variants in GBA

The frequency of *GBA* variants in DLB patients varies between populations, ranging from 3.5% in a cohort of neuropathological confirmed DLB cases from the United States[20] to 33% in a clinical DLB cohort of Ashkenazi Jews, a population in which variants in *GBA* are overrepresented[21,69]. Variations in this frequency are due to differences in population and diagnostic criteria (clinically diagnosed patients versus pathologically diagnosed patients), as well as differences in research methods (e.g. genotyping of specific variants versus whole coding region) and selection criteria of identified variants (e.g. inclusion of all variants

versus rare pathogenic variants). Currently, the largest multicenter study has reported a disease-associated rare variant frequency of 7.5% in 721 clinically diagnosed DLB patients, compared to 0.97% in 1962 controls.[19]

Recent studies show that DLB patients carrying disease-associated *GBA* variants may have a different clinical disease course than those without such variants.[19,21,43] A study among Ashkenazi Jews with DLB has shown more severe motor complaints, REM-sleep behavior disorders, and cognitive dysfunction in carriers than in non-carriers.[21] Furthermore, several studies have shown an earlier age of disease onset and death in DLB patients carrying a rare *GBA* variant than in non-carriers.[19,21,43]

Many rare and common disease-associated variants in *GBA* have been found in DLB and PD patients.[19-21,39-41,43,70] The risk of disease or a particular phenotype is dependent on the type of disease-associated variant.[69,71]

Rare variants in SNCB

The *SNCB*: p.P123H variant has been found in a family with DLB[32,34] (Table 1) and the p.V70M variant in a DLB patient without affected family members[32] (Supplementary Table 1). The clinical diagnosis of DLB was pathologically confirmed in the patient with the p.P123H variant, but did not fully cosegregate in his family. The p.P123H and p.V70M variants have not been found in other DLB patients, PD patients, or in 331 control individuals from the population of the affected patients.[32] Possible pathogenicity is supported by the location of the variants, as they reside in highly conserved regions.[32] It is also supported by research in transgenic mice expressing the p.P123H variant, where progressive neurodegeneration was observed.[72] Further studies are necessary to replicate these findings.

Other rare variants with unclear pathogenicity

Other genes harboring rare variants with unclear pathogenicity which have been associated with DLB are *parkin* (*PARK2*)[13,24], *PTEN induced putative kinase 1* (*PINK1*)[24], *granulin* (*GRN*)[24,44,45], *triggering receptor expressed on myeloid cells 2* (*TREM2*)[45], *charged multivesicular body protein 2B* (*CHMP2B*)[13], *sequestosome* (*SQSTM1*)[13], *microtubule-associated protein tau* (*MAPT*)[24] and *prion protein* (*PRNP*)[46] (Supplementary Table 1). Defects in these genes have previously been associated with other neurodegenerative diseases, such as PD and frontotemporal dementia (FTD), but have only sporadically been found in DLB patients. Rare variants in *coiled-coil-helix-coiled-coil-helix domain containing 2* (*CHCHD2*)[47], *eukaryotic translation initiation factor 4 gamma 1* (*EIF4G1*)[13] and *GRB10 interacting GYF protein 2* (*GIGYF2*)[13] have also incidentally been associated with DLB (Supplementary Table 1). However, the role of these genes in neurodegeneration has not yet been conclusively established.

Common variants in APOE

The *APOE* ε4 allele has repeatedly been associated with DLB.[11,13,24,27-30] Its frequency in DLB patients varies between studies, but is approximately 30% in Caucasian DLB patients[11,13,24,27,28,30] in comparison to 14% in Caucasian controls free of neurodegenerative and neuropsychiatric diseases[73].

The effect of the *APOE* ε4 allele on different levels of Lewy and AD pathology was studied in 640 patients with dementia and 269 cognitively normal controls. This study showed that *APOE* ε4 allele carriers have an increased risk of both AD and Lewy pathology: *APOE* ε4 allele carriers had a 13-fold increased risk of developing a dementia with Lewy and AD pathology, a 10-fold increased risk of developing a dementia with only AD pathology, and a 6-fold increased risk of developing a dementia with Lewy pathology in comparison with non-carriers.[28] These findings suggest that the *APOE* ε4 allele may be a larger risk factor for dementia with both Lewy and AD pathology than for dementia with AD pathology only, and may contribute to the development of dementia through mechanisms unrelated to AD pathology.[28] Studies investigating the influence of the *APOE* ε4 allele on disease course report that DLB patients carrying an *APOE* ε4 allele have a shorter survival than non-carriers.[11,13] In contrast to *APOE* ε4 allele carriers, *APOE* ε2 allele carriers have a reduced risk of developing DLB in comparison with non-carriers.[74]

Common variants in MAPT

Recently, an association study was performed in which *MAPT* haplotypes were investigated in clinically diagnosed DLB patients (n=431), patients with Lewy pathology and a high likelihood of clinical DLB (n=347), and in individuals without dementia or movement disorders (n=1049). The H1G haplotype was associated with a higher risk of DLB in comparison with controls (2.8% vs. 1.0%, OR=2.2). In line with findings in PD, the H2 haplotype was associated with a lower risk of DLB in comparison with controls (20.9% vs. 23.6%, OR=0.8).[31,75] Other *MAPT* haplotypes (e.g. H1C and H1P) have also been linked to PD, PDD or AD[76-78], which suggests that different haplotypes may increase the risk of a specific phenotype[31]. Replication of findings in larger cohorts of patients is necessary to validate these genetic associations.

Other common variants

The largest association study to date not only showed an association between the *APOE* locus, but also between the *SNCA* and *scavenger receptor class B member 2* (*SCARB2*) loci and DLB. This study investigated 54 genomic regions, that were previously implicated in PD or AD, and was conducted in 788 clinically diagnosed DLB cases, of which 85% were neuropathologically confirmed, and 2624 controls.[12] Interestingly, the associations observed in this study for the *SNCA* and *SCARB2* loci were different than those previously found for PD.[12] This suggests that these loci may play a subtle different role in these diseases. In addition, a common variant in *butyrylcholinesterase* (*BuChE*) has been associated with a decreased risk of DLB (n=174) in comparison with controls (n=86) in a recent study.[79]

Genetic overlap between DLB, AD and PD

Genetic research in DLB has mainly focused on genes associated with PD and AD. Because of this biased approach, nearly all disease-associated variants found in DLB overlap with those associated with PD and AD (Figure 2). To assess the genetic overlap in a more unbiased way, an analysis of genetic correlation of DLB, PD and AD was performed.[80] In this study, a genome-wide genotyping was conducted on 788 clinically diagnosed DLB cases of which 85% were neuropathologically confirmed, 804 PD cases, and 959 clinically diagnosed AD cases. The proportion of variance explained by all single nucleotide polymorphisms (SNPs) for DLB was 0.31, for AD 0.60 and for PD 0.28. When comparing DLB with PD and DLB with AD for these SNPs, a correlation of 0.36 and 0.58 was found, respectively. No genetic correlation between PD and AD was found. Limitations of this study were the relatively small sample size, the inclusion of common risk loci only, and possible selection bias of the array. Nevertheless, the results of this study are interesting. First, the study suggests a larger overlap between DLB and AD than between DLB and PD. Secondly, the absence of an association between AD and PD indicates that the mechanisms underlying the association of DLB with AD and with PD are completely different. Finally, this study also suggests that, although genetic factors overlap with AD and PD, it is likely that DLB-specific genetic factors exist.[80]

Taken together, studies in DLB show that identical genetic defects are associated with several phenotypes in the PD-DLB-AD spectrum. This suggests that these diseases share some underlying mechanisms, but that other genetic or non-genetic factors may also play a role. There are also indications that specific genetic defects within an identical gene are linked to different clinical and pathological features in the PD-DLB-AD spectrum. This in turn suggests that the underlying mechanisms may be similar, but are, for example, dependent on the severity of the defect, which could be reflected in the level of pathology and clinical symptoms. Only a few studies indicate a role of DLB-specific genetic factors in the development of the disease.

However, a number of limitations to these current genetic studies may influence their quality. These, and the outlook for future research are discussed in the next two sections.

Limitations of current genetic studies

When interpreting genetic findings in DLB, a number of aspects have to be taken into account. First, genetic studies have often been performed in clinically and pathologically heterogeneous groups of patients. In the past, different diagnostic and pathologic criteria and nomenclature for DLB were used, which has made comparison of studies problematic. Applying the revised consensus diagnostic criteria of McKeith et al., 2005[2] led to greater homogeneity of diagnosis. Although these criteria have a high specificity (90%), the low sensitivity (54%)[81] is the main reason why a definite DLB diagnosis can only be made after autopsy. However, accurate clinical information is still required to differentiate between DLB

and late stage PD or PDD, as these diseases cannot be reliably distinguished based on the neuropathology alone.[82] Currently, the differentiation between DLB and PDD is based on the 'one year rule', in which DLB is diagnosed when dementia presents before or within a year after the onset of parkinsonism.[2] PDD is diagnosed when dementia starts more than one year after an established PD diagnosis. This is a somewhat artificial rule for differing between two conditions on the same spectrum of Lewy body diseases.[83] However, the rule is still applied as it increases the homogeneity of the study population and research comparability.[2] Only a selected group of genetic studies have been performed on patients with both a clinical and a neuropathological diagnosis of DLB. Misdiagnosis is possible in those studies with a lack of either neuropathological confirmation or detailed clinical information.

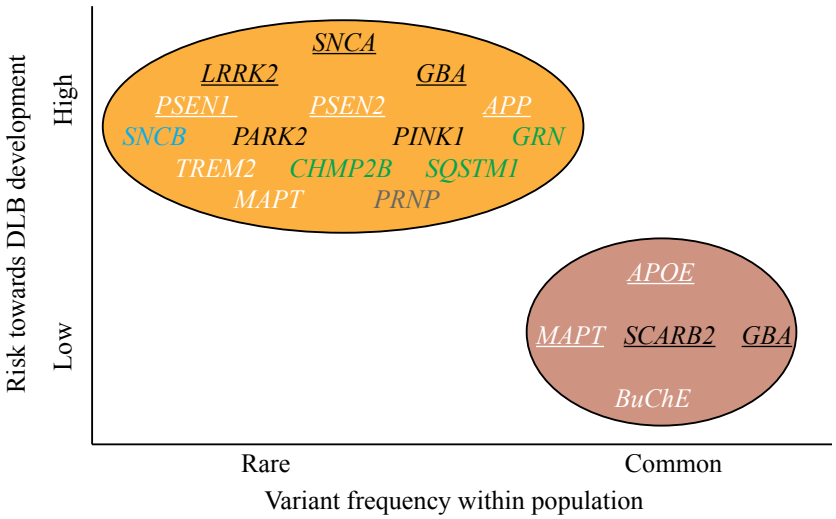

Figure 2: Variant risk versus variant frequency for DLB.
Genes previously associated with PD, AD, FTD and Creutzfeldt-Jacob's disease, and no other neurodegenerative disease are depicted in black, white, green, grey, and blue respectively. Evidence for the association between genes and DLB is stronger for those genes that are underlined than not underlined.

Secondly, only a few genetic findings from family studies have been reported. In addition, in these studies segregation of the variant of interest was not always studied and genetic analysis was not always performed in the DLB patients, but instead in affected relatives.

Thirdly, the pathogenicity of many of the identified variants is unclear, which leads to the question whether these variants are directly related to DLB or are just coincidental findings. Support of pathogenicity can, for example, be obtained from well-designed studies in which the prevalence of a specific variant is significantly higher in affected individuals in comparison with controls. Well-established *in vitro* or *in vivo* functional studies on specific variants can also support the claim of pathogenicity.[84] Genetic studies with large sample sizes

of affected and control patients are scarce for DLB. Furthermore, very few functional and replication studies have been performed in DLB.

Future research

To increase our understanding of the role of genetics in DLB, we believe that future genetic studies should focus on the optimization of conventional research methods, on the implementation of next generation sequencing technologies, and on the combination of different research modalities.

Optimization of conventional genetic research methods

Ideally, future genetic studies should focus on DLB patients with both a clinical and pathological diagnosis of DLB, while taking the amount of coincidental pathological findings (especially AD pathology) into account. For this, multicenter studies are essential to ensure substantial patient numbers. A systematic analysis of genes previously associated with neurodegenerative diseases in a large group of these patients can lead to a better understanding of the role of these genes in DLB. Another interesting, but biased, approach is to select genes that play a role in pathways related to PD and AD. In addition to single nucleotide variants, structural variants, such as copy-number variants and inversions, must also be taken into account.[85] However, to find new genetic determinants, unbiased research is necessary. Unbiased linkage studies may in general be a powerful tool in the search for new disease-causing defects, but these are not feasible for the identification of new genetic determinants in DLB given the rarity and often small size of DLB-families. GWA studies may be a source of unbiased information about new loci containing common variants with small to moderate effect sizes. Yet, functional and replication studies are still necessary when resolving the role of genetic defects with unclear pathogenicity in DLB.

Next generation sequencing

Whole exome or genome sequencing studies which provide the opportunity to screen simultaneously for genetic variants in the entire exome or genome[37,38], have not yet been reported for DLB. Applying these techniques in homogeneous, well-phenotyped groups, such as families, patients with a similar disease course or identical amount of (coincidental) pathology, may increase the chances of finding new genetic variants for DLB. Whole exome and genome sequencing may especially help in the identification of genetic variants with a low frequency and intermediate effect size that are hard to detect with conventional research techniques.[37]

Multimodal approach

Applying combinations of different research modalities, such as genomics, transcriptomics and proteomics, may further increase the chance of finding new

genetic determinants for DLB. A challenge when using these techniques is the selection of the variable of interest from a large amount of data. However, combining these techniques can reduce the number of potential disease-associated genetic variants. The application of whole exome sequencing in combination with proteomic research has been successful in identifying new genetic variants in several diseases.[86,87] For example, Wong et al., 2015[86] reported a rare autosomal dominant neurodegenerative disorder comprising of parkinsonism and dementia in a large family, and found the causal mutation in *protein kinase cAMP-dependent type I regulatory subunit beta* (*PRKAR1B*) by combining linkage analysis, whole exome sequencing, and proteomics.

Currently, four proteomic studies[88-91] and one transcriptomic study[92] have been performed in DLB patients (Supplementary Table 2). The results have not directly led to a reduction in the number of potential disease-associated genetic variants, as the proteomic profile found in these studies varies substantially because of the use of different inclusion criteria, research techniques, and different specimens (e.g. blood versus brain tissue) at different stages of the disease.

In future, multimodal research, the combination of unbiased genomic analysis and proteomics may prove particularly valuable for researching diseases like DLB, in which easily discernable and large pathological inclusions may provide a large amount of material for proteomic analyses.

Conclusion

To date, rare variants in *GBA* and the *APOE* ε4 allele are the strongest known risk factors for DLB. Defects in other genes have also been found in DLB patients. However, the risk profile of many of these defects has yet to be determined. Most of the genes associated with DLB overlap with genes associated with PD and AD, which suggests common neurobiological mechanisms for these diseases. However, because of the different phenotypes, other genetic and non-genetic factors may also play a role. Because no large unbiased genetic studies have been performed, it is likely that multiple DLB-specific determinants still have to be identified. The combination of different research modalities, such as next generation sequencing and proteomics may help in the identification of these determinants. The search for DLB-specific genetic determinants is important as it will give us a better understanding of the pathophysiology of DLB and its relation with PD and AD. This in turn could ultimately lead to the development of disease modifying treatments.

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Supplementary Table 1: List of rare disease-associated variants identified in unrelated DLB patients.

Gene	Protein change	Observed in number of DLB patients	Number of DLB patients in study	Diagnosis based on clinical criteria	Diagnosis based on pathological criteria	Observed in number of control population	Number of control patients in study	Reference
SNCA	duplication	1	99	99	1	NA	NA	[24]
LRRK2	p.G2019S	1	772	417	355	0	1790	[42]
GBA	several	54	721	721	443	19	1962	[19]
		18	95	0	95	1	32	[39]
		14	301	0	301	3	381	[20,40]
		8	35	0	35	NA	NA	[41]
		11	33	33	0	NA	NA	[21]
		5	97	97	50	0	115	[43]
PARK2	p.A46S, p.R275W	2	99	99	1	NA	NA	[24]
	p.R275W/p.G430D	1	91	91	91	NA	NA	[13]
PINK1	p.P138L, p.M318L, p.S499C	3	99	99	1	NA	620	[24]
GRN	p.L261I/p.R433W	1	99	99	1	NA	620	[24]
	Leu271LeufsX10	1	45	45	0	0	120	[44]
	several	7	58	58	58	12	380	[45]
TREM2	p.R62H	7	58	58	58	5	368	[45]
CHMP2B	p.I29V	1	91	91	91	NA	NA	[13]
SQSTM1	p.A33V, p.P27L	2	91	91	91	NA	NA	[13]
MAPT	p.R221Q	1	99	99	1	0	620	[24]
PRNP	p.M232R	1	1	0	1	NA	NA	[46]
Not confirmed genes								
SNCB	p.V70M	1	43	43	20 + 9*	0	331	[32]
CHCHD2	several	6	610	>1	610	1	717	[47]
EIF4G1	p.M1134V	1	91	91	91	NA	NA	[13]
GIGYF2	p.S1029C, p.S66T	2	91	91	91	NA	NA	[13]

* Lewy pathology in at least the patient or a family member with DLB. DLB: dementia with Lewy bodies, NA: not available, as genetic analysis in a control group was not performed or reported.

Supplementary Table 2: Overview of proteomic and transcriptomic studies in DLB.

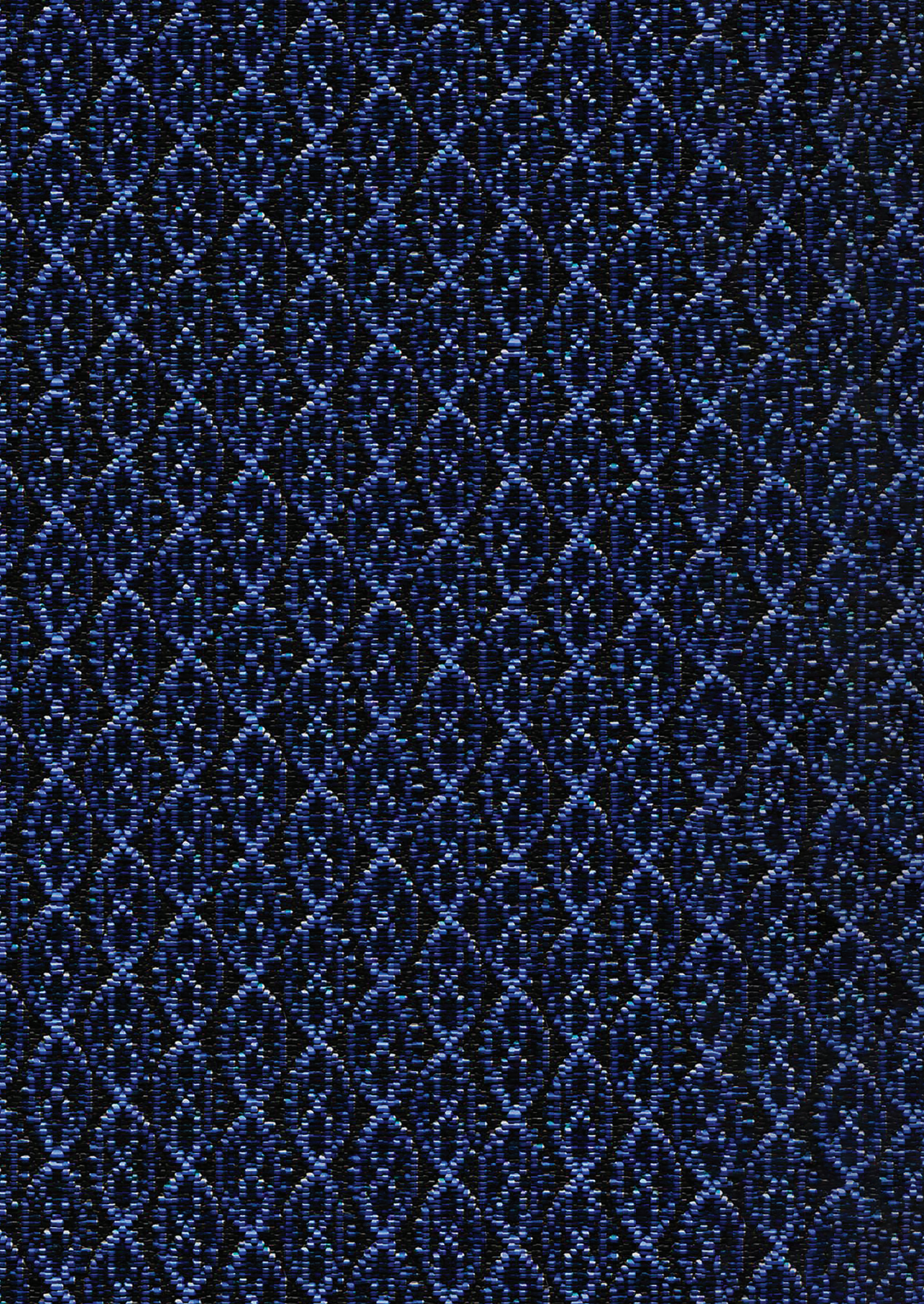
Proteomics

Patients	Based on clinical diagnosis	Pathologically confirmed	Control group	Specimen	Method	Number of proteins	Number of differentially expressed proteins	Validated proteins	Reference
dementia (n=6)	yes	cortical Lewy bodies	no	2500 Lewy bodies (pooled)	laser dissection microscopy, liquid chromatography-tandem mass spectrometry	296	n.a.	heat shock cognate 71 kDA	[88]
DLB (n=5), PD (n=10), AD (n=10)	yes	only DLB, AD	n=10 (age matched, healthy volunteers)	CSF	isobaric tagging for relative and absolute protein quantification, multidimensional chromatography, tandem mass spectrometry	1539	380 (DLB versus controls, proteomic changes >50% as compared to controls)	apoC1, t-cadherin	[89]
DLB/PDD (n=10)	yes	no	n=15 (age matched, not further specified)	CSF	label-free liquid chromatography-tandem mass spectrometry	>1000	22 (DLB/PDD vs controls with fold change >2)	osteopontin, ubiquitin carboxy-terminal hydrolase L1, chitinase-3-like protein 1	[90]
DLB (n=30), AD (n=30)	yes	no	n=28 (healthy controls, age differed significantly with disease groups)	serum	matrix-assisted laser desorption/ionization time of flight mass spectrometry	146 peptides	14 (DLB vs controls with fold change >1,5)	none	[91]

Transcriptomics

Patients	Clinical diagnosis	Pathological confirmed	Control group	Biomaterial	Method	Number of differentially expressed genes	Reference
DLB (n=8)	yes	yes	n=10	anterior cingulate cortex	gene expression profiling	367 downregulated	[92]

DLB: dementia with Lewy bodies, PD: Parkinson's disease, AD: Alzheimer's disease, PDD: Parkinson's disease dementia, CSF: cerebrospinal fluid, n.a.: not applicable.



Part 2

Familial aggregation in dementia with Lewy bodies

2.1

Chapter 2.1

Family history is associated with phenotype in dementia with Lewy bodies

Leonie J.M. Vergouw*, Brechje Bosman*, Marleen van de Beek, Mariet Salomé, Susanne E. Hoogers, Inger van Steenoven, Gerwin Roks, Vincenzo Bonifati, John C. van Swieten, Afina W. Lemstra, Frank Jan de Jong

* Shared first author

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Abstract

It is currently unknown whether patients with dementia with Lewy bodies (DLB) with relatives with dementia or Parkinson's disease (PD) (familial DLB patients) have a different phenotype than sporadic DLB patients. In this study, we aimed to examine disease onset, rate of cognitive decline, survival and Alzheimer's disease (AD) biomarkers in patients with familial DLB (n=154) and sporadic DLB (n=137), using linear mixed model analysis and Cox regression analysis, among others. Familial patients had a shorter survival (8.0 years) and more often elevated CSF AD biomarkers (47%) than sporadic patients (9.0 years; p=<0.001; 30%, p=0.037). Our findings suggest that genetic factors are important in DLB and that the identification of new genetic factors will probably improve the prediction of prognosis.

Introduction

Dementia with Lewy bodies (DLB) is one of the most common forms of degenerative dementia in the older population.[1] DLB is diagnosed when dementia is accompanied by at least two of the following four core clinical features: parkinsonism, visual hallucinations, fluctuating cognition, and rapid eye movement (REM)-sleep behavior disorders (RBD). DLB can also be diagnosed based on dementia with one core clinical feature, in the presence of reduced dopamine transporter uptake in the basal ganglia, abnormal ^{123}iodine-MIBG myocardial scintigraphy, or polysomnographic confirmation of RBD.[2] Symptoms of DLB are not specific to the disease, but overlap with clinical features of Parkinson's disease (PD), PD dementia (PDD) and Alzheimer's disease (AD).[1] The distinction between DLB and PDD is most challenging, and based on differences in time between onset of dementia and parkinsonism. In PDD, dementia occurs in the context of well-established PD as opposed to DLB, in which dementia occurs before or concurrently with parkinsonism.[2] In addition, pathological and genetic features are also shared between DLB, PD(D) and AD.[1,3,4] For example, Lewy bodies containing the α-synuclein protein are the pathological hallmark of DLB, but are also observed in PD(D)[1], and genetic factors, such as the *APOE* ε4 allele and *GBA* variants, are risk factors for DLB as well as for AD and PD(D), respectively[3,4]. However, genetic risk factors for AD and PD seem to explain a part of the total phenotypic variance in DLB only.[5-7]

Recent studies have indicated that genetic factors play an important role in DLB. The heritable component of DLB has even been estimated at 60%.[7] Families with multiple DLB patients have rarely been described.[3,8] However, it has been reported that siblings of DLB patients are at higher risk of developing DLB compared to siblings of AD patients.[9] Furthermore, DLB patients more often have a family history of PD or dementia than controls.[10,11] This finding supports the notion that DLB, PD and dementia share, at least partially, the same genetic factors. This in turn might lead to shared molecular pathways and possibly similar phenotypes.

The *APOE* ε4 allele has been associated with a shorter survival in DLB[12-14] and disease-associated genetic variants in *GBA* have been associated with an earlier age of onset and death in DLB[15-17]. However, it is currently unknown whether DLB patients with relatives with dementia or PD (familial DLB) have a different phenotype than sporadic DLB patients. The main aim of this study is to examine the role of family history, used as a proxy of genetic factors, in relation to disease onset, rate of cognitive decline, survival and AD biomarkers. The secondary aim of this study is to explore the aforementioned features in DLB patients with relatives with dementia or PD to examine if their phenotype is more similar to AD or PD.

Materials and methods

Patients and study design

This is a retrospective study in which demographic and clinical data were collected from patients' medical records. Information on the occurrence of dementia and/or PD in first-degree relatives was based on medical records (41%) or a standardized assessment (59%), using a questionnaire or an additional patient/family member interview. A nation-wide registration system containing demographic data about all Dutch citizens was consulted to obtain information about dates of death (collected until December 2018).

A total of 291 patients with probable DLB according to the criteria of Mckeith et al. (2005)[18] were enrolled from three hospitals in the Netherlands (Elisabeth-TweeSteden Hospital, Tilburg; Erasmus Medical Center, Rotterdam; Amsterdam University Medical Center, Amsterdam). Patients visited the outpatient clinics of the Neurology departments between 2000 and 2018 and were diagnosed by expert neurologists. Dopamine transporter uptake scans were performed in 128 patients, and were used in the diagnostic process. Familial patients were defined as patients with at least one first-degree relative with dementia or PD. Sporadic patients were defined as patients without first-degree relatives with dementia or PD. Patients were excluded from the study when no dementia or PD was diagnosed in relatives together with no information on the occurrence of the other disease in relatives (e.g. no relatives with PD and no information of the occurrence of dementia, or vice versa), and when no information on family history was available at all. The distribution of the different groups according to family history is depicted in Supplementary Figure 1.

The study was performed according to the ethical principles of the Declaration of Helsinki and was approved by the local ethics committees (Elisabeth-TweeSteden Hospital: MEC-2016-608, L0318.2016; Erasmus Medical Center: MEC-2015-304, MEC-2016-608; Amsterdam University Medical Center: MEC-2016-061, MEC-2017-2116).

Outcome measures

Several clinical features and AD biomarkers (i.e. the presence of medial temporal lobe atrophy (MTA)[19], a CSF tau/amyloid-$\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) ratio of >0.52[20] and ≥1 *APOE* ε4 allele(s)[21]), were explored with respect to family history in DLB.

Age of onset and type of first symptom were based on anamnestic information from the patient or family members. We categorized the type of first symptom into cognitive decline, parkinsonism and hallucinations. Cognitive decline included descriptions of memory impairment and executive function impairment. Parkinsonism was based on bradykinesia with muscular rigidity, rest tremor or postural instability[22] or when parkinsonism was noted in the medical files in absence of more specific information. Rate of cognitive decline was based on the available Mini-Mental State Examination (MMSE) scores[23]. Survival was defined as the time between age of onset and death. Magnetic resonance imaging (MRI)

scans were evaluated visually and rated according to the MTA scale[19] by radiologists for clinical purposes. CSF was collected by lumbar puncture in polypropylene tubes (Starstedt, Nümbrecht, Germany). Levels of $A\beta_{1-42}$, total tau and p-tau were measured with commercially ELISAs (Innotest®, Fujirebio, Gent, Belgium). *APOE* genotyping was performed using the LightCycler *APOE* mutation detection method (Roche Diagnostics GmbH, Mannheim, Germany) after genomic DNA extraction.

Data analysis

Differences in sex, age of onset, type of first symptom and *APOE* genotype between familial and sporadic DLB were explored using the χ^2 test, Fisher's Exact Test, Fisher-Freeman-Halton test, independent Student t test or Mann-Whitney U test, where appropriate. We analyzed the MTA score and CSF tau/$A\beta_{1-42}$ ratio of >0.52 (CSF AD biomarkers) between the groups using linear and logistic regression, respectively, with time between age of onset and date of MRI or lumbar puncture as covariate to correct for possible confounding by disease stage. Linear mixed model analysis were performed to assess changes in MMSE levels over time, while accounting for the correlation between the repeated measurements of each patient. The model included time since first MMSE, family history and an interaction effect between time and family history to assess differences in the course of cognitive decline between groups. All MMSEs which were administered within 6 months from the previous MMSE were removed, because of possible learning effects. In the random effect structure, covariance type was set on unstructured and a random intercept was included. Differences in survival between familial and sporadic DLB were explored using Kaplan-Meier analysis and Cox regression analysis. We included sex, age of onset and study center to correct for possible confounding

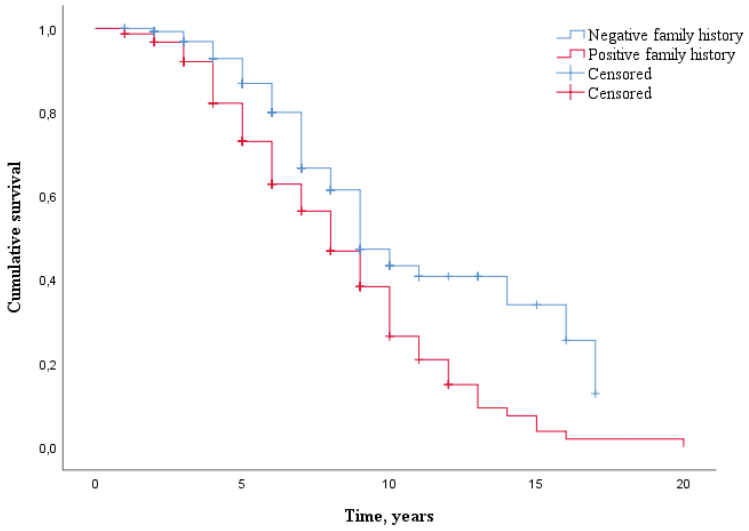

Figure 1: Survival distribution (Kaplan-Meier curve) of DLB patients with a negative family history as compared to DLB patients with a positive family history.

in the linear mixed model analysis and the Cox regression analysis.

In addition, differences in phenotype between DLB patients with a family history of either dementia or PD were explored. Statistical analyses were performed as described above.

Statistical significance for all tests was established at $p<0.05$ (two-tailed). The data was analyzed using SPSS software (Version 24).

Results

Differences in phenotype between patients with familial and sporadic DLB

The 154 familial DLB patients and the 137 sporadic DLB patients did not differ in sex, age of onset, type of first symptom (Table 1) or rate of cognitive decline (Table 2). Significantly more familial DLB patients had elevated CSF biomarkers for AD (47%), compared to those with sporadic DLB (30%, $p=0.039$). This finding remained significant after correction for time between age of onset and date of lumbar puncture ($p=0.037$, Table 1). In addition, a borderline significant result was found concerning the *APOE* ε4 allele, with a higher frequency of this allele in familial patients (65%) compared to sporadic patients (51%, $p=0.069$). MTA score was not different between the groups (Table 1).

Differences in survival between patients with familial and sporadic DLB

A total of 154 patients (53.1%) were deceased with a median survival of 7.0 years (IQR 5.0–9.0). Uncorrected survival analysis showed that patients with familial DLB had a significantly shorter survival (median 8.0 years, SE 0.51) compared to patients with sporadic DLB (median 9.0 years, SE 0.63, $p=<0.001$, Table 2 and Figure 1). This finding remained significant after correction for sex, age of onset and study center (HR 1.64, 95% CI: 1.16-2.31, $p=0.005$, Table 2).

Differences in phenotype between DLB patients with a family history of either dementia or PD

A total of 84 patients had a family history of either dementia (n=69) or PD (n=15) (Supplementary Figure 1). Parkinsonism was more prevalent as first symptom in DLB patients with a family history of PD (33%) compared to the patients with a family history of dementia (6%, $p=0.008$). These groups did not differ in sex, age of onset (Supplementary Table 1) or rate of cognitive decline (Supplementary Table 2).

In addition, there were no significant differences in the presence of the *APOE ε4* allele, CSF AD biomarkers and MTA score between the groups (Supplementary Table 1).

Differences in survival between DLB patients with a family history of either dementia or PD

A total of 43 patients (51.2%) were deceased with a median survival of 8.0 years (IQR 5.0–10.0). There were no differences in survival between DLB patients with relatives with dementia as compared to DLB patients with relatives with PD (Supplementary Table 2).

Table 1: Demographic features, clinical features and biomarkers in the total study group, and group differences.

	Total (n=291)	Familial DLB (n=154)	Sporadic DLB (n=137)	*p* value
Study center				0.011*
Elisabeth-TweeSteden Hospital	35 (12%)	24 (16%)	11 (8%)	
Erasmus Medical Center	54 (19%)	20 (13%)	34 (25%)	
Amsterdam University Medical Center	202 (69%)	110 (71%)	92 (67%)	
Sex, male	232 (80%)	122 (79%)	110 (80%)	0.82
Age of onset, years	66.0 (7.6)	66.6 (8.4)	65.3 (6.7)	0.14
First symptom				
Cognitive decline	245 (84%)	133 (86%)	112 (82%)	0.28
Parkinsonism	29 (10%)	14 (9%)	15 (11%)	0.60
Hallucinations	17 (6%)	7 (5%)	10 (7%)	0.32
MTA score (average of right and left) (n=199[98;101])	1 (0.5-1.5)	1 (0.5-1.5)	1 (0-1.1)	0.10[a]
CSF tau/$A\beta_{1\text{-}42}$ ratio >0.52 (n=169[83;86])	65 (38%)	40 (47%)	25 (30%)	0.037[a]*
APOE ε4 carrier (n=160[76;84])	94 (59%)	55 (65%)	39 (51%)	0.069

Values are presented as mean (SD), median (IQR) or n (%). DLB: dementia with Lewy bodies, PD: Parkinson's disease, CSF:cerebrospinal fluid, MTA: medial temporal lobe atrophy, $A\beta_{1\text{-}42}$: amyloid-$\beta_{1\text{-}42}$,[a] corrected for time between age of onset and date of MRI or date of lumbar puncture, * $p<0.05$.

Table 2: Statistical models regarding rate of cognitive decline and survival in the total study group.

Rate of cognitive decline		**β**	**SE**		**95% CI**	***p* value**	
Uncorrected (Linear mixed model analysis)	Baseline	-0.47	0.59		-1.63-0.70	0.43	
	Change over time	-0.0090	0.017		-0.041-0.024	0.59	
With correction for sex, age of onset, and study center (Linear mixed model analysis)	Baseline	-0.34	0.59		-1.50-0.81	0.56	
	Change over time	-0.0091	0.016		-0.042-0.023	0.59	
Survival		**Median[a]**	**SE**		**95% CI**	***p* value**	
Uncorrected (Kaplan-Meier analysis)	Sporadic DLB	9.0	0.63		7.77-10.23	<0.001*	
	Familial DLB	8.0	0.51		7.00-9.00		
		B	**SE**	**HR**	**95% CI**	***p* value**	**Z-score**
With correction for sex, age of onset, and study center (Cox regression analysis)		0.50	0.18	1.64	1.16-2.31	0.005*	5.71

[a]Time between age of onset and death,* $p<0.05$.

Discussion

The main finding of this study is that patients with familial DLB have a shorter survival than patients with sporadic DLB. We also found a higher percentage of familial DLB patients with elevated AD biomarkers in their CSF compared to sporadic DLB patients. Several longitudinal studies in DLB showed that concomitant AD pathology is associated with a

higher mortality.[24-26] The shorter survival in familial DLB compared to sporadic DLB may be due to concomitant AD pathology in familial DLB, which is reflected in a higher CSF tau/ $A\beta_{1-42}$ ratio in familial patients compared to sporadic patients. Genetic factors, such as the *APOE* ε4 allele, contribute to the presence of concomitant AD pathology.[25,27] Interestingly, although only borderline statistically significant, a higher frequency of the *APOE* ε4 allele in familial patients compared to sporadic patients was seen. This suggests that genetic factors are associated with survival, possibly by influencing neuropathology.

Previous studies have shown several possible risk factors for a shorter survival in DLB, such as a the presence of a fluctuating cognition, hallucinations at onset and a low MMSE score.[14,26] Other variables have also been reported to be associated with survival, but are contradictory between different studies (e.g female and male sex, early and late age of onset).[14,26] There were no significant differences in these clinical characteristics between familial and sporadic DLB patients in our study (data not shown for a fluctuating cognition and MMSE score). This might indicate that family history has an effect on survival independent of these clinical characteristics.

In addition, we found parkinsonism to be a more frequent presenting symptom in patients with a positive family history of PD than in patients with a positive family history of dementia. This might be based on more 'pure' Lewy pathology in patients with relatives with PD, and mixed pathology (Lewy pathology and AD pathology) in patients with relatives with dementia. However, this finding could also be based on recall bias due to familiarity with PD symptoms. Other AD or PD features were equally distributed between patients with a positive family history of dementia or PD, respectively. These results should be interpreted with caution due to the relative small number of patients in the group with relatives with PD and the presence of non-AD dementias in the group with relatives with dementia.

About half of the DLB patients in this study had at least one first-degree relative with dementia or PD. This is in line with previous studies, in which a positive family history for dementia was observed in 39-44% and for PD in 10-24% of patients with DLB.[10,11] Nonetheless, the percentages that we found could be an overestimation as patients with insufficient information on family history were not taken into account. These patients might be less likely to have relatives with relevant diseases. However, the most important limitation of this study is its retrospective character. Furthermore, information on family history and disease onset was based on anamnestic information, and may have introduced a recall bias. In addition, the patients in this study were predominantly male (80%), which may not be a good representation of the general DLB population.[28] However, our main finding (familial DLB patients have a shorter survival than sporadic DLB patients) stayed significant after correction for sex. At last, the DLB diagnoses in this study were not pathologically confirmed.

Strengths of this study include its large sample size and the enrollment of patients from university medical centers as well as from a general hospital. The latter increases the generalizability of our findings.

In conclusion, we are the first to report that DLB patients with a positive family history

of dementia or PD have a shorter survival than DLB patients with a negative family history of these diseases. This suggests that genetic factors contribute to disease course, possibly by influencing the amount of concomitant AD pathology, which is supported by our data. Future studies are necessary to identify which genetic and other contributing factors are accountable for our findings. This knowledge will lead to a better understanding of the pathophysiology of the disease and the overlap with AD and PD(D), and will probably improve the prediction of prognosis.

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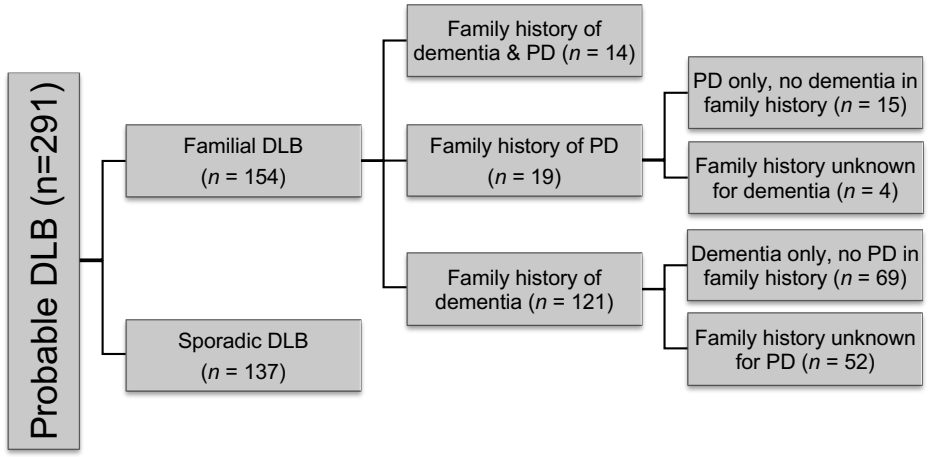
Supplementary Information



Supplementary Figure 1: Distribution of different groups according to family history. Family members with PDD were assigned to the subcategory 'Family history of dementia & PD', and family members with DLB were assigned to the subcategory 'Family history of dementia'. DLB: dementia with Lewy bodies, PD: Parkinson's disease, PDD: Parkinson's disease dementia.

Supplementary Table 1: Differences in demographic features, clinical features and biomarkers between DLB patients with either a family history of dementia or a family history of PD.

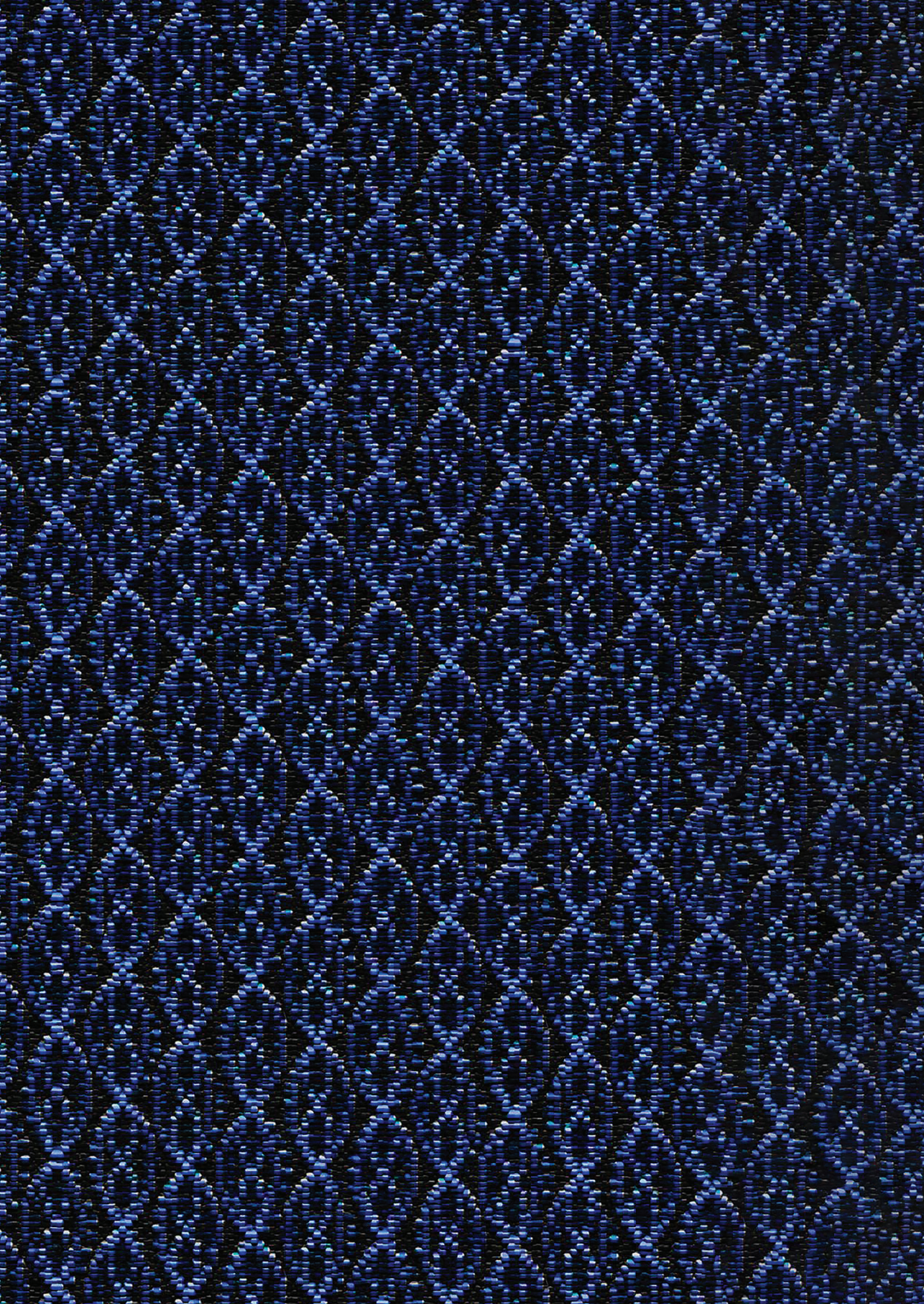
	Family history of dementia only (n=69)	**Family history of PD only (n=15)**	***p* value**
Study center			0.72
Elisabeth-TweeSteden Hospital	11 (16%)	3 (20%)	
Erasmus Medical Center	7 (10%)	2 (13%)	
Amsterdam University Medical Center	51 (74%)	10 (67%)	
Sex, male	55 (80%)	13 (87%)	0.73
Age of onset, years	64.6 (8.0)	64.5 (8.7)	0.99
First symptom			
Cognitive decline	60 (87%)	10 (67%)	0.12
Parkinsonism	4 (6%)	5 (33%)	0.008*
Hallucinations	5 (7%)	0 (0%)	0.58
MTA score (average of right and left) (n=51;9)	1 (0.5-1.5)	1 (0-1.3)	0.24[a]
CSF tau/Aβ_{1-42} ratio >0.52 (n=43;6)	22 (51%)	1(17%)	0.13[a]
APOE ε4 carrier (n=43;6)	28 (65%)	4 (67%)	1.00

Data are presented as mean (SD), median (IQR) or n (%). DLB: dementia with Lewy bodies, PD: Parkinson's disease, CSF: cerebrospinal fluid, MTA: medial temporal lobe atrophy, Aβ_{1-42}: amyloid-β_{1-42}, [a] corrected for time between age of onset and date of MRI or date of lumbar puncture, * p<0.05.

Supplementary Table 2: Statistical models regarding rate of cognitive decline and survival in the group of DLB patients with either a family history of dementia or a family history of PD.

Rate of cognitive decline		**β**	**SE**		**95% CI**	***p* value**	
Uncorrected	Baseline	0.67	1.51		-2.33-3.66	0.66	
(Lineair mixed model analysis)	Change over time	-0.022	0.045		-0.11-0.067	0.62	
With correction for sex, age of onset, and study center (Linear mixed model analysis)	Baseline	0.34	1.50		-2.63-3.31	0.82	
	Change over time	-0.015	0.045		-0.10-0.074	0.73	
Survival		**Median**[a]	**SE**		**95% CI**	***p* value**	
Uncorrected (Kaplan-Meier analysis)	Family history of dementia only	10.0	0.65		8.72-11.28	0.97	xx
	Family history of PD only	10.0	1.98		6.12-13.88		xx
		B	**SE**	**HR**	**95% CI**	***p* value**	**Z-score**
With correction for sex, age of onset, and study center (Cox regression analysis)		0.20	0.46	1.23	0.50-3.03	0.66	2.17

[a] Time between age of onset and death, * $p<0.05$.



Part 3

Known genes associated with dementia with Lewy bodies

3.1

Chapter 3.1

Familial dementia with Lewy bodies: a comprehensive analysis of genes involved in Parkinson's or Alzheimer's disease

Leonie J.M. Vergouw, Marialuisa Quadri, Inger van Steenoven, Hanneke Geut, Guido J. Breedveld, Netherlands Brain Bank, Wilma D.J. van de Berg, Annemieke J.M. Rozemuller, Afina W. Lemstra, John C. van Swieten, Vincenzo Bonifati, Frank Jan de Jong

Abstract

Introduction

The genetic architecture of dementia with Lewy bodies (DLB) is poorly understood, but overlaps with Parkinson's disease (PD) and Alzheimer's disease (AD). Here, we performed a comprehensive analysis of genes known to be involved in PD or AD in a series of familial DLB patients, to gain more insight into their involvement in DLB.

Methods

We included twenty clinically diagnosed DLB patients with a positive family history. Neuropathological confirmation was available in 55% of the patients. By whole exome sequencing, we investigated variants in all the genes known to be involved in PD and AD. Copy number variants in selected genes, the *C9orf72* repeat expansion and the *APOE* risk allele were also investigated. Last, genotype-phenotype correlations of *GBA* variants and the *APOE* ε4 allele were explored.

Results

We identified five *GBA* variants (p.D140H, p.E326K, p.T369M, p.N370S, and p.R463C) in 35% of our patients. Furthermore, 20% of the patients carried a rare variant with unknown significance in *LRRK2*, *PARK2*, *ABCA7* or *SORL1*. The *APOE* ε4 allele frequency was 38%.

Conclusion

GBA variants and the *APOE* ε4 allele were frequently found. This confirms the importance of these two genes in DLB and the genetic overlap between DLB, PD and AD. Future genetic stratification may help to predict disease course and to select patients for disease-modifying clinical trials.

Introduction

Dementia with Lewy bodies (DLB) is one of the most prevalent types of dementia[1] and is characterized by cognitive decline in combination with parkinsonism, visual hallucinations, cognitive fluctuations, and REM-sleep behavior disorders[2,3].

Although its heritable component has been estimated to be 36% in a recent cohort of 1743 DLB patients[4], only few large association or exome sequencing studies have been reported[4-9]. These studies have shown that specific variants in *glucocerebrosidase* (*GBA*) and the *apolipoprotein E* (*APOE*) risk allele, which have previously been associated with Parkinson's disease (PD)[10] and Alzheimer's disease (AD) respectively[11,12], are also prominent risk factors for DLB. Moreover, variants in genes previously associated with PD, such as *α-synuclein* (*SNCA*) and *leucine-rich repeat kinase 2* (*LRRK2*), or in genes previously associated with AD, such as *presenilin 1* (*PSEN1*), *presenilin 2* (*PSEN2*) and *amyloid precursor protein* (*APP*), have been identified in some patients with DLB by family studies.[13] However, large families with multiple DLB patients are rarely reported, and genetic research has mainly been conducted in families with different phenotypes ranging within the DLB-PD-AD spectrum. Studies based on highly-selected series of pathologically-confirmed familial DLB patients, might facilitate the identification of novel genetic variants involved in the disease etiology.[13]

In this study, we aimed to further elucidate the genetic underpinnings of DLB and its overlap with PD and AD by studying a series of familial DLB patients.

Materials and Methods

Participants

Two groups of patients with clinically and/or pathologically diagnosed DLB and a positive family history (at least one first- or second-degree relative with DLB, PD or dementia) were studied.

The first group includes all patients (n=10) who visited the outpatient clinic of the Erasmus Medical Center in Rotterdam or the VU University Medical Center in Amsterdam between 2015 and 2017, who received a clinical diagnosis of probable DLB[2], and also had at least one first-degree relative affected with DLB, PD or dementia available for genetic studies. Pathological confirmation of the disease was available in one index patient. When possible, affected and non-affected relatives were also included in the study.

The second group includes all patients (n=10) from the Netherlands Brain Bank (NBB), who donated their brain between 1999 and 2013, had pathologically confirmed DLB (Braak α-synuclein stage: >4[14]; Braak neurofibrillary tangle stage: <4[15]), a retrospective clinical diagnosis of probable DLB[2] and a positive family history. Nine of these patients had at least one first-degree relative affected with DLB, PD or dementia, whereas one patient had one second-degree affected relative.

Clinical features were collected and genetic analyses were performed in all the 20 index patients. When possible, co-segregation studies were also carried out.

This study was approved by the relevant Medical Ethical Authorities, and all patients or their legal representative signed informed consent for use of clinical records, DNA, and pathological data for research purposes.

Sample preparation, exome capture and exome sequencing

Genomic DNA was isolated from blood in group 1 and from blood or cerebellar tissue in group 2 using standard methods. Whole exome sequencing (WES) was performed using the Nimblegen SeqCap EZ Exome v.2.0 44Mb kit (Roche Nimblegen, Inc., Madison, WI) on a HiSeq2000 sequencer (paired-end 2x100). Reads were aligned to the human reference genome (hg19) using the Burrows-Wheeler alignment[16] tool and processed using Picard (http://broadinstitute.github.io/picard) and the Genome Analysis Toolkit (GATK)[17] following standard procedures[18]. Single nucleotide variations were determined using GATKs Haplotype Caller and annotated using ANNOVAR[19].

Filtering

Non-synonymous, stop-gain or stop-loss variants in exons, and variants near splice sites in genes with a well-established involvement in PD or AD were extracted from the WES data (Supplementary Table 1). Subsequently, variants were selected based on a minor allele frequency (MAF) of <1% in the ExAC-NFE (Exome Aggregation Consortium-Non Finnish Europeans) database and the GoNL (Genome of the Netherlands) database. For *GBA*, variants were followed up regardless the allele frequency reported in public databases to include both rare and common variants.

Sanger sequencing

Variants that fulfilled our filtering criteria were validated by Sanger sequencing (Supplementary Text; Supplementary Table 2). Exons and exon-intron boundaries with low coverage (<10 reads) in the WES analysis were also Sanger sequenced to exclude false-negative calls.

Both DNA strands were directly sequenced using the Big Dye Terminator chemistry ver.3.1 (Applied Biosystems) on an ABI3130/ABI3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Subsequent analysis was performed with SeqScape (ver.2.6).

Copy number analysis

Multiplex Ligation-dependent Probe Amplification (MLPA) was performed to analyze copy dosage of *SNCA*, *PARK2*, *PARK7*, *PINK1* and *APP*. The P051-D1 Parkinson and P170-C2 APP MLPA kits (MRC Holland) were used according to the manufacturer's protocol. Subsequent analysis was performed on an ABI3130/ABI3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The MLPA data were analyzed using GeneMarker Ver.2.4.0 (SoftGenetics, State College, PA, USA).

Table 1: Demographic, clinical and pathological features.

	Total (n=20)	Group 1 (n=10)	Group 2 (n=10)
Probable DLB	20 (100%)	10 (100%)	10 (100%)
Sex, male	15 (75%)	8 (80%)	7 (70%)
Age of onset, years	66.5 (8.6)	64.4 (8.5)	68.6 (8.6)
First symptom			
Parkinsonism	9 (45%)	2 (20%)	7 (70%)
Cognition	10 (50%)	8 (80%)	2 (20%)
Hallucinations	1 (5%)	0 (0%)	1 (10%)
Parkinsonism during disease course	18 (90%)	8 (80%)	10 (100%)
Disease duration[a], years (n=2;10)	5.8 (2.9)	5.5 (4.9)	5.8 (2.8)
Deceased	12 (60%)	2 (20%)	10 (100%)
Family history			
DLB	3 (15%)	0 (0%)	3 (30%)
PD/PDD	3 (15%)	3 (30%)	0 (0%)
AD/dementia	9 (45%)	2 (20%)	7 (70%)
Combination	5 (25%)	5 (50%)	0 (0%)
Autopsied	11 (55%)	1 (10%)	10 (100%)
Braak α-synuclein stage			
5	2 (18% [b])	1 (100% [b])	1 (10%)
6	9 (82% [b])	0 (0% [b])	9 (90%)
Braak neurofibrillary tangle stage			
1	2 (18% [b])	0 (0% [b])	2 (20%)
2	3 (27% [b])	0 (0% [b])	3 (30%)
3	5 (45% [b])	1 (100% [b])	4 (40%)
4	1 (9% [b])	0 (0% [b])	1 (10%)
CERAD			
O	2 (18% [b])	0 (0% [b])	2 (20%)
A	2 (18% [b])	0 (0% [b])	2 (20%)
B	4 (36% [b])	1 (100% [b])	3 (30%)
C	3 (27% [b])	0 (0% [b])	3 (30%)
Thal phase			
0	1 (9% [b])	0 (0% [b])	1 (10%)
1	1 (9% [b])	1 (100% [b])	0 (0%)
2	1 (9% [b])	0 (0% [b])	1 (10%)
3	5 (45% [b])	0 (0% [b])	5 (50%)
4	3 (27% [b])	0 (0% [b])	3 (30%)

Data are presented as mean (SD) or n (%). [a] first symptom until death. [b] in percentage of patients who were autopsied. CERAD: Consortium to Establish a Registry for Alzheimer's Disease, DLB: dementia with Lewy bodies, PD: Parkinson's disease, PDD: Parkinson's disease dementia, AD: Alzheimer's disease.

C9orf72 repeat expansion analysis

A previously described repeat-primed PCR assay[20] was used to screen for the presence of a pathogenic chromosome 9p21 GGGGCC hexanucleotide repeat expansion. Fragment length analysis was performed on an ABI3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Subsequent data were analyzed using GeneMarker Ver.2.4.0 (SoftGenetics, State College, PA, USA). A pathogenic *C9orf72* expansion was defined as more than 30 repeats.[20] Positive controls were included in this analysis.

Table 2: Genetic variants.

Position	dbSNP 142 accession number	Nucleotide change	Amino acid change	Variant type	Allele frequency GoNL (alleles)	Allele frequency ExAC-NFE (alleles)	Allele frequency GnomAD (alleles)	Allele frequency HEX (alleles)	Functional predictions: pathogenic (total)	Group 1 Clinically diagnosed and positive family history (n=10)	Group 2 Pathologically diagnosed and positive family history (n=10)
PD genes											
GBA											
Exon 6	rs147138516	c.535G>C	p.D140H (p.D179H)	missense	0.10% (1)	0.01% (7)	0.01% (20)	.	4 (10)	2 (family 1 and 2)	1
Exon 10	rs76763715	c.1226A>G	p.N370S (p.N409S)	missense	.	0.36% (242)	0.23% (623)	0.31% (3)	7 (10)	0	1
Exon 11	rs80356771	c.1504C>T	p.R463C (p.R502C)	missense	.	0.01% (4)	0.01% (37)	0.15% (1)	9 (10)	1 (family 3)	0
Exon 9	rs2230288	c.1093G>A	p.E326K (p.E365K)	missense	2.30% (23)	1.20% (798)	1.01% (3025)	0.94% (9)	3 (9)	2[a] (family 1 and 2)	2[a]
Exon 9	rs75548401	c.1223C>T	p.T369M (p.T408M)	missense	1.10% (11)	0.98% (627)	0.62% (1702)	0.92% (6)	3 (9)	0	1
LRRK2											
Exon 40	rs201012950	c.5870G>T	p.R1957L	missense	.	0.00% (2)	0.01% (22)	.	5 (10)	1 (family 4)	0
PARK2											
Exon 3	rs55774500	c.245C>A	p.A82E	missense	0.40% (4)	0.71% (471)	0.35% (978)	0.10% (1)	1 (9)	1 (family 5)	0
AD genes											
ABCA7											
Exon 5	novel	c.403G>A	p.A135T	missense	.	.	.	.	4 (9)	1 (family 6)	0
SORL1											
Exon 13	rs545522170	c.1729T>C	p.S577P	missense	0.10% (1)	0.00% (3)	0.00% (11)	.	5 (10)	0	1

[a] Both the p.D140H and p.E326K were observed in two patients from group 1 and one patient from group 2. PD: Parkinson's disease, AD: Alzheimers's disease, GoNL: Genome of the Netherlands, ExAC-NFE: Exome Aggregation Consortium-Non Finnish Europeans, GnomAD: Genome Aggregation Database (non-Finnish Europeans), HEX: Healthy Exomes > 60 years, Alzforum. Transcript references: NM_001005742 (*GBA*); NM_198578 (*LRRK2*), NM_004562 (*PARK2*), NM_019112 (*ABCA7*), NM_003105 (*SORL1*).

APOE ε risk allele analysis

Genotypes for allelic variants rs7412 and rs429358 were determined using Taqman Allelic Discrimination (Supplementary Text). Signals were read with the Taqman 7900HT (Applied Biosystems Inc.) and analyzed using sequence detection system 2.3 software (Applied Biosystems Inc.).

Statistical analysis

Differences in demographic and clinical features between genetic variant carriers and non-carriers were analysed with the independent Student's t-test, χ^2-test or Fisher's Exact Test where appropriate. Statistical analyses were performed in IBM SPSS Statistics 21.0 for Windows (SPSS Inc., IL, USA). *P* values of <0.05 were considered statistically significant.

Results

Patient features

Table 1 shows the demographic, clinical and pathological features of the patients. The mean age at disease onset of the total group (75% male) was 66.5 ± 8.6 years. The mean disease duration was 5.8 ± 2.9 years. Fifteen percent of the patients had relatives with DLB, 15% relatives with PD or PD dementia (PDD), 45% relatives with dementia including AD, and 25% relatives with a combination of the previously mentioned diseases. All patients that were autopsied (n=11) had a Braak α-synuclein stage[14] of ≥5 and a Braak neurofibrillary tangle stage[15] of ≤4. CERAD[21] was B or C in 64% of the patients and Thal phase[21] was ≥3 in 73% of the patients.

Genes associated with PD

Seven heterozygous variants in three genes associated with PD (*GBA*, *LRRK2* and *PARK2*) were identified in 45% (9/20) of patients (Table 2; Figure 1).

Glucocerebrosidase

GBA heterozygous variants were observed in seven of the 20 patients (35%). Two patients carried a single rare variant (p.N370S or p.R463C), three patients carried a rare variant (p.D140H) in combination with a more common variant (p.E326K), and two patients carried a single, more common variant (p.E326K or p.T369M). The observed rare variants cause Gaucher disease (in homozygous or compound heterozygous state) and act as confirmed PD risk factors (in single heterozygous state). The more common variants do not cause Gaucher disease (in homozygous or compound heterozygous state), but still act as a mild risk factors for PD (in single heterozygous state)[22, 23] (Table 2).

Co-segregation analysis was possible for three DLB patients from group 1. In family 1, the proband (II-1) carried both the p.D140H and p.E326K variant, whereas his half-brother with DLB (II-3) carried the p.E326K variant only. In family 2, both the proband (III-2) and

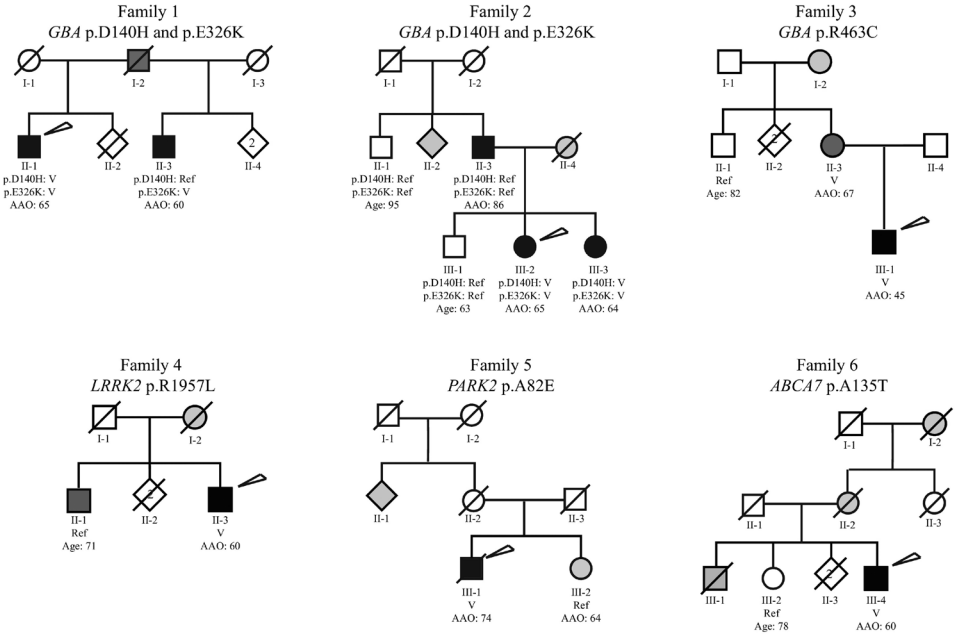


Figure 1: Pedigrees of patients with genetic variants identified.
A circle represents a female patient; a square represents a male patient; a diamond represents a patient with unknown sex; black symbols indicate patients affected by DLB; dark grey symbols indicate patients with PD or PDD and light grey symbols indicate patients with AD or unspecified dementia. V: variant, Ref: reference (wild type genotype), AAO: age at onset, DLB: dementia with Lewy bodies, PD: Parkinson's disease, PDD: Parkinson's disease dementia, AD: Alzheimer's disease.

her sister with DLB (III-3) carried these two variants, but not the father with DLB (II-3). In family 3, the proband (III-1) and his mother with PDD (II-3) carried the p.R463C variant, which was absent in his unaffected uncle (II-1) (Figure 1).

Other genes associated with PD

In two patients we detected single rare variants with unknown significance in *LRRK2* (p.R1957L) and in *PARK2* (p.A82E).

The proband of family 4 (II-3) carried the *LRRK2* p.R1957L variant, which was absent in his brother with PD (II-1) (Figure 1). The MAF of this variant is 0.01% in the GnomAD (Genome Aggregation Database (non-Finnish Europeans)), whereas it is absent in GoNL. It is predicted to be possibly pathogenic by M-CAP and by four out of nine other *in-silico* programs (Table 2; Supplementary Table 3).

The proband of family 5 (III-1) carried a heterozygous *PARK2* p.A82E variant, which was absent in his sister with dementia (III-2) (Figure 1). The MAF of this variant is 0.35% in the GnomAD and is predicted to be benign in nearly all *in-silico* programs (Table 2; Supplementary Table 3).

No dosage abnormalities in *SNCA*, *PARK2*, *PARK7* or *PINK1* were found by MLPA

analysis. This analysis was not possible in one patient from the second group, because of low DNA quality.

Genes associated with AD

Two heterozygous variants in two genes associated with AD (*ABCA7* and *SORL1*) were identified in 10% (2/20) of patients (Table 2; Figure 1).

The proband of family 6 (III-4) carried the *ABCA7* p.A135T variant, which was absent in his unaffected sister (III-2) (Figure 1). This variant is not present in public databases, and is predicted to be possibly pathogenic by four out of nine *in-silico* programs.

The p.S577P variant in *SORL1* is found in one patient from group 2. This variant has a MAF of 0.004% in the GnomAD and is predicted to be pathogenic by half of the *in-silico* programs.

The allele frequency for *APOE* ε2, ε3 and ε4 were 0%, 63% and 38%, respectively (Table 3). Furthermore, no dosage abnormalities in *APP* and no pathogenic *C9orf72* repeat expansions were found. The MLPA analysis was not possible in one patient from the second group, because of low DNA quality. Supplementary Table 4 shows an overview of the pathological findings in the genetic variant carriers.

Table 3: *APOE* ε allele frequencies.

***APOE* allele**	**Total (n=20)**	**Group 1 (n=10)**	**Group 2 (n=10)**
ε2	0 (0%)	0 (0%)	0 (0%)
ε3	25 (63%)	15 (75%)	10 (50%)
ε4	15 (38%)	5 (25%)	10 (50%)

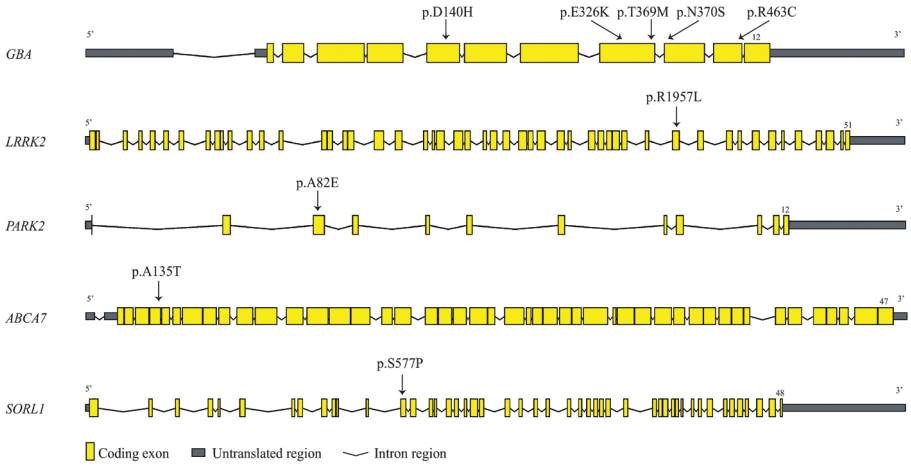


Figure 2: Gene structures.
Locations of the variants found in this study are indicated. Genes are displayed with 5'to 3'orientation and the number of exons is displayed. Transcript references: NM_001005742 (*GBA*), NM_198578 (*LRRK2*), NM_004562 (*PARK2*), NM_019112 (*ABCA7*) and NM_003105 (*SORL1*).

Table 4: Demographic and clinical features in *GBA* variant carriers and non-carriers and *APOE* ε4 allele carriers versus non-carriers.

	***GBA* variant carriers (*GBA*+) (n=7)**	**Non *GBA* variant carriers (*GBA*-) (n=13)**	***p* value**	***APOE* ε4 allele carriers (*APOE* ε4+) (n=13)**	**Non *APOE* ε4 allele carriers (*APOE* ε4-) (n=7)**	***p* value**
Sex, male	5 (71%)	10 (77%)	1.0	10 (77%)	5 (71%)	1.0
Age of onset, years	61.4 (9.6)	69.2 (6.9)	0.05*	68.8 (8.1)	62.3 (8.3)	0.11
First symptom			0.77			1.0
Parkinsonism	4 (57%)	5 (38%)		6 (46%)	3 (43%)	
Cognition	3 (43%)	7 (54%)		6 (46%)	4 (57%)	
Hallucination	0 (0%)	1 (8%)		1 (8%)	0 (0%)	
Parkinsonism during disease course	7 (100%)	11 (85%)	0.52	11 (85%)	7 (100%)	0.52
Disease duration[a], years (n=4;8|12;2)	8.5 (2.1)	4.4 (2.3)	0.01*	4.9 (2.3)	10.0 (1.4)	0.02*
Family history[b] (n=4;8|8;3)			0.15			0.006*
PD/PDD	2 (67%)	1 (13%)		0 (0%)	3 (100%)	
AD/dementia	1 (33%)	7 (87%)		8 (100%)	0 (0%)	

Data are presented as mean (SD) or n (%). [a] first symptom until death. [b] only first-degree relatives, a family history of both PD/PDD and AD/dementia were not taken into account.* Statistically different between groups. PD: Parkinson's disease, PDD: Parkinson's disease dementia, AD: Alzheimer's disease.

Genotype-phenotype correlations

Demographic and clinical features in *GBA* variant carriers (*GBA*+) and non-carriers (*GBA*-), and *APOE* ε4 allele carriers (*APOE* ε4+) and non-carriers (*APOE* ε4-) are displayed in Table 4. Given the small number of patients, we consider the following results as exploratory. In the *GBA*+ group, age of onset was earlier and disease duration longer than in the *GBA*- group (61.4 ± 9.6 years vs 69.2 ± 6.9 years, *p* value 0.05; 8.5 ± 2.1 years vs 4.4 ± 2.3 years, *p* value 0.01).

When stratifying on the presence of at least one *APOE* ε4 allele, disease duration was significantly shorter in the *APOE* ε4+ group than in the *APOE* ε4- group (4.9 ± 2.3 years vs 10.0 ± 1.4 years, *p* value 0.02). Furthermore, age of onset was significantly earlier in the *GBA*+ group compared to the *APOE* ε4+ group (58.5 ± 8.2 (n=4) vs 69.8 ± 7.2 (n=10), *p* value 0.03; patients with both a *GBA* variant and the *APOE* ε4 allele were excluded).

Interestingly, the *APOE* ε4+ group had significantly more often a family history of AD or dementia than with PD or PDD in contrast to the *APOE* ε4- group (family history AD/dementia; PD/PDD: 100%; 0% vs 0%; 100%, *p* value 0.006). When comparing the type of family history between the *GBA*+ group and the *APOE* ε4+ group, a family history of PD or PDD was more often found in *GBA*+ patients and a family history of AD or dementia in *APOE* ε4+ patients (family history PD/PDD; AD/dementia: 100%; 0% (n=2) vs 0%; 100% (n=7), *p* value 0.03; patients with both a *GBA* variant and the *APOE* ε4 allele were excluded).

Supplementary Figure 1 gives a graphical overview of the first-degree relatives with DLB (in black), PD or PDD (in grey), and AD or dementia (in white) referred among the *GBA*+ (Figure 1a) and *APOE* ε4+ patients (Figure 1b).

Discussion

Rare and common *GBA* variants and the *APOE* ε4 allele were frequently observed in this cohort of familial DLB patients. Furthermore, rare variants with unknown significance were found in *LRRK2*, *PARK2*, *ABCA7* and *SORL1*. This study provides further evidence that genes associated with PD and AD are also involved in the etio-pathogenesis of DLB, and suggests that genetic risk factors also influence disease course.

GBA variants have been associated with PD in several studies[10], but they are also increasingly recognized as risk factors for DLB[7]. We found *GBA* variants in 35% of our cohort of familial patients. Twenty-five percent of our patients carried a rare PD-associated variant in *GBA*. *GBA* variant frequencies in DLB range between studies due to differences in population, diagnostic criteria and genetic analysis methods.[13] The largest multicenter study published so far, reported a rare variant frequency of 7.5% in 721 clinically or pathologically diagnosed DLB patients and 0.97% in 1962 controls.[7] The higher frequency of these rare *GBA* variants in our study could represent a higher load of genetic factors in our patients who were selected on the basis of a positive family history, and the proportion of pathologically confirmed patients with a low Braak neurofibrillary tangle stage (<4), which has been associated with higher *GBA* variant frequencies.[24] The *GBA* variants observed in our study have all been previously associated with PD and DLB.[7,10,22,24-26] PD and DLB-associated variants in *GBA* can be divided into mild and severe[27], depending on the severity of the phenotype associated in Gaucher disease patients who carry these variants in homozygous state. A recent, large study of 1000 Ashkenazi-Jews with PD showed odds ratios for PD as different as 2.2 for mild variants (e.g. p.D140H and p.N370S) and 10.3 for severe variants (e.g. p.R463C). Furthermore, age of PD onset was significantly earlier in carriers of severe variants than those who carried mild variants.[27] In our study, we found one severe variant (p.R463C) and two mild variants (p.D140H and p.N370S). The combination of the p.D140H and E326K variant was found in three of our patients. Interestingly, the p.D140H and E326K variant combination has, together with the p.R463C variant, been associated with an increased cognitive decline in PD patients.[28] Further studies are necessary to determine if these variants are more frequently associated with DLB than PD.

The *APOE* ε4 allele is the most frequent, known risk factor for AD[11,12], and it has repeatedly been associated with DLB[4,8,9]. In our cohort, the mean *APOE* ε4 allele frequency (38%) is comparable to that previously reported in DLB (approximately 30%).[13,29-31]

We found several rare variants of unknown significance in *LRRK2*, *PARK2*, *ABCA7* and *SORL1*. The *LRRK2* p.R1957L variant is of interest, because it is extremely rare in public databases (MAF: <0.01%), is predicted to be pathogenic by half of the *in-silico* programs,

and is located in the kinase domain like the G2019S mutation, which is the most common genetic cause of PD.[32]

Pathogenic repeat expansions in *C9orf72* have been found in clinically and pathologically diagnosed AD patients[33], but not in pathologically confirmed DLB patients[34,35]. Our results are in line with the above-mentioned studies, as no pathogenic *C9orf72* repeat expansions were identified.

Given the small number of patients, exploratory analyses were performed concerning genotype-phenotype correlations of *GBA* variants and the *APOE* ε4 allele. These results must be interpreted with caution. Our analysis showed that the age of onset was earlier in the *GBA*+ group versus *GBA*- group, which has been reported before in previous studies.[7,36,37] Disease duration was longer in the *GBA*+ group versus *GBA*- group. Previous studies reported a similar disease duration between these groups.[36,37] Likewise, as in previous studies[5,8] disease duration was shorter in the *APOE* ε4+ group compared to the *APOE* ε4- group, which may be explained by a higher burden of AD concomitant pathology in *APOE* ε4+ patients.[9,38,39] Furthermore, we showed that *GBA*+ patients had more often a family history of PD or PDD than AD or dementia, and that *APOE* ε4+ patients had more often a family history of AD or dementia than PD or PDD. Based on these results, we speculate that, when looking at the PD-DLB-AD spectrum, DLB *GBA*+ patients are pathophysiological more related to the PD spectrum, and DLB *APOE* ε4+ patients to AD.

Our data support a genetic overlap between DLB, PD and AD, and they suggest that these diseases share common molecular mechanisms. This study confirms that the same genetic variant (e.g. *GBA* variants or the *APOE* ε4 allele) can be associated with different phenotypes in the DLB-PD or DLB-AD spectrum; this suggests that other genetic or non-genetic factors are important for the final resulting clinical and pathological phenotypes. Interestingly, studies also suggest[4,28,40] that specific variants within the same gene (for example the *GBA* p.D140H and E326K variant combination) may be more often associated with a specific phenotype within this spectrum. We also described new variants in DLB patients located in genes known to be involved in PD and AD, but whether they play a role in the disease development in our patients remains unknown.

This study has limitations. The total number of patients is low; clinical data were collected retrospectively in half of them; and postmortem examination was not performed in all. Particularly, the results of our genotype-phenotype correlations should be interpreted with caution, and further studies are necessary to support our findings.

Conclusion

This study confirms that *GBA* variants and the *APOE* ε4 allele play important roles in familial DLB. These findings also support the notion that genetic overlaps exist between DLB, PD and AD. Future stratification based on *GBA* and *APOE* variants may help to predict disease course and select patients for clinical trials of disease-modifying drugs.

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Supplementary Information

PCR protocol Sanger sequencing

Our standard PCR mix contained 2 µl PCR buffer, 1.6 µl dNTPs 2.5 mM, 1 µl forward primer 10 µM, 1 µl reverse primer 10 µM, 0.1 µl Fast-start taq or Platinum taq, 2 µl DNA (12.5 ng/µl) and 12.3 µl H_2O per reaction. An adjusted PCR mix was used to amplify fragment 1 of *GBA* and contained 10 µl TAKARA GCII buffer, 1.6 µl dNTPs 2.5 mM, 1 µl forward primer 10 µM, 1 µl reverse primer 10 µM, 0.1 µl TAKARA LA taq, 2 µl DNA (12.5 ng/µl) and 4.4 µl H_2O per reaction. PCR cycling consisted of initial denaturation for 5.5 minutes at 96°C, 31 or 32 cycles with denaturation for 30 seconds at 96°C, annealing for 30 seconds at 60°C and extension for 1.5 minutes at 72°C, and a final extension for 5.5 minutes at 72°C.

PCR protocol APOE ε risk allele analysis

Reactions were performed in a 384-wells format in a total volume of 2 µl containing 2 ng DNA, 1x genotyping assay (Thermo Fisher Scientific) and 1x genotyping master mix (Thermo Fisher Scientific). PCR cycling consisted of initial denaturation for 10 minutes at 95°C, and 40 cycles with denaturation of 15 seconds at 96°C and annealing and extension for 60 seconds at 60°C.

Supplementary Table 1: Genes with a well-established involvement in Parkinson's disease or Alzheimer's disease.

	Chromosome	Start	End	Mode of inheritance
PD genes				
GBA	1	155204239	155214653	autosomal dominant / risk factor
LRRK2	12	40618813	40763087	autosomal dominant
MAPT	17	43971702	44105700	autosomal dominant
SNCA	4	90645250	90759447	autosomal dominant
VPS35	16	46693589	46723144	autosomal dominant
RAB39B	X	154487526	154493852	X-linked
DJ1	1	8021714	8045342	autosomal recessive
DNAJC6	1	65720133	65881552	autosomal recessive
PARK2	6	161768590	163148834	autosomal recessive
PINK1	1	20959948	20978004	autosomal recessive
AD genes				
ABCA7	19	1040102	1065571	autosomal dominant / risk factor
APP	21	27252861	27543446	autosomal dominant
PSEN1	14	73603143	73690399	autosomal dominant
PSEN2	1	227057885	227083804	autosomal dominant
SORL1	11	121322912	121504471	autosomal dominant / risk factor
APOE	19	45409039	45412650	risk factor

Coordinates based on human reference genome (hg19; NCBI). PD: Parkinson's disease, AD: Alzheimer's disease.

Supplementary Table 2: Primers for Sanger sequencing.

Primer name	Sequence	Use
GBA_fragment1_Fa	cctaaagttgtcacccatac	PCR
GBA_fragment1_Ra	agcagacctaccctacagttt	PCR + sequencing
GBA_fragment3_Fa	tgtgtgcaaggtccaggatcag	PCR + sequencing
GBA_fragment3_Ra	accacctagaggggaaagtg	PCR
GBA_exon6_F	cccaggagcccaagttccc	sequencing
GBA_exon9_R	ctggacaggaagggcttctg	sequencing
GBA_exon10_F	ctgacctacccacagctgc	sequencing
GBA_exon10_R	tgatgggactgtcgacaaag	sequencing
GBA_exon11_F	gagagccagggcagagcctc	sequencing
GBA_exon11_R	tgagtcacccaaaccattgc	sequencing
LRRK2_exon40_F	gaagaaatggaaagtttgctatgatcc	PCR + sequencing
LRRK2_exon40_R	tcagggaaatggtagttttcatcc	PCR + sequencing
PARK2_exon3_F	tgtaactgctgtgggcaaagg	PCR + sequencing
PARK2_exon3_R	caaagtactccacctacagtgatgtctcc	PCR + sequencing
ABCA7_exon5_F	CAACTTCAACGACTCCCTgtgagc	PCR + sequencing
ABCA7_exon5_R	GGAGACTGCTTGGTTGGTTGAGG	PCR + sequencing
SORL1_exon13_F	cctttgccttagagactttcactgc	PCR + sequencing
SORL1_exon13_R	tcaattacctccctatgctttttgc	PCR + sequencing

[a] As *GBA* has a pseudogene which is very similar, two different sets of primers were used. Large fragments, specific for *GBA*, were amplified using PCR, after which other primers were used to be able to sequence the smaller regions of interest.

Supplementary Table 3: *In-silico* pathogenicity predictions of genetic variants.

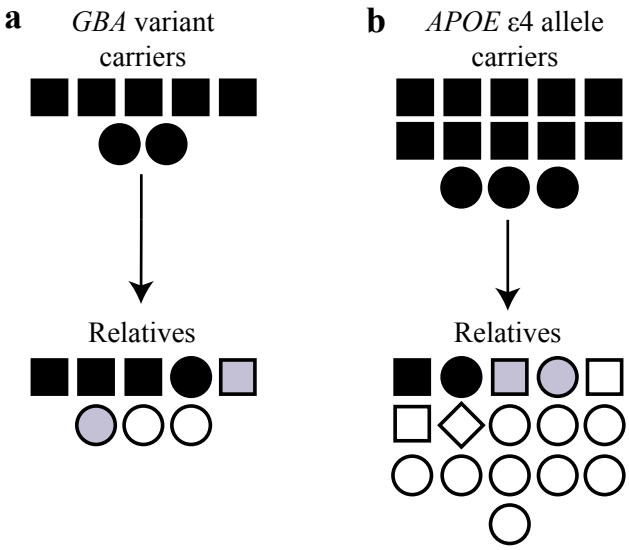
							Variant-effect predictions software (scores)									
Gene	**Chromo-some**	**Genomic position**	**Reference**	**Alternative**	**Nucleotide change**	**Amino acid change**	**GERP**	**SIFT**	**Polyphen2 HDIV**	**Polyphen2 HVAR**	**LRT**	**Mutation Assessor**	**FATHMM**	**Radial SVM**	**LR**	**CADD phred**	**M-CAP**
GBA																	
	1	155208361	C	G	c.535G>C	p.D140H (p.D179H)	2.62	**T** (0.16)	**P** (0.712)	**P** (0.653)	**N** (0.007)	**L** (1.88)	**D** (-5.8)	**D** (0.903)	**D** (0.925)	11.51	**PP** (0.404)
	1	155205634	T	C	c.1226A>G	p.N370S (p.N409S)	3.53	**D** (0.02)	**P** (0.607)	**B** (0.292)	**D** (0)	**L** (1.57)	**D** (-5.77)	**D** (1.036)	**D** (0.903)	18.4	**PP** (0.746)
	1	155204987	G	A	c.1504C>T	p.R463C (p.R502C)	2.11	**D** (0.02)	**D** (0.999)	**D** (0.932)	**N** (0.003)	**M** (2.34)	**D** (-5.84)	**D** (1.058)	**D** (0.968)	19.35	**PP** (0.917)
	1	155206167	C	T	c.1093G>A	p.E326K (p.E365K)	3.67	**T** (0.43)	**B** (0.03)	**B** (0.043)	**N** (0.075)	**L** (1.01)	**D** (-5.77)	**D** (0.675)	**D** (0.85)	11.06	**NA**
	1	155206037	G	A	c.1223C>T	p.T369M (p.T408M)	3.57	**T** (0.12)	**B** (0.275)	**B** (0.113)	**N** (0.006)	**L** (1.5)	**D** (-5.71)	**D** (0.902)	**D** (0.879)	11.4	**NA**
LRRK2																	
	12	40728881	G	T	c.5870G>T	p.R1957L	4.85	**T** (0.26)	**P** (0.834)	**P** (0.635)	**N** (0.003)	**L** (0.975)	**D** (-3.23)	**D** (0.574)	**D** (0.725)	21.1	**PP** (0.123)
PARK2																	
	6	162683724	G	T	c.245C>A	p.A82E	-0.77	**T** (0.81)	**B** (0.026)	B (0.007)	**N** (0.652)	**N** (0.695)	**D** (-2.83)	**T** (-0.805)	**T** (0.265)	5.08	**NA**
ABCA7																	
	19	1042163	G	A	c.403G>A	p.A135T	1.08	**T** (0.71)	**B** (0.035)	**B** (0.01)	**NA**	**N** (0.75)	**D** (-2.13)	**D** (0.075)	**D** (0.78)	8.46	**PP** (0.038)
SORL1																	
	11	121414300	T	C	c.1729T>C	p.S577P	5.8	**T** (0.09)	**D** (0.999)	**D** (0.996)	**D** (0)	**L** (1575)	**T** (1.04)	**T** (-0.747)	**T** (0.222)	28	**PP** (0.044)

M-CAP does not provide pathogenicity scores, when the mean allele frequency is not < 1% in all databases. GERP: Genomic Evolutionary Rate Profiling, SIFT: Sorting Intolerant From Tolerant, PolyPhen2 HDIV: Polymorphism Phenotyping version 2 human diversity, PolyPhen2 HVA: Polymorphism Phenotyping version 2 human variation, LRT: Likelihood Ratio Test, FATHMM: Functional Analysis Through Hidden Markov Models; SVM: Support Vector Machine, LR: Logistic Regression, CADD: Combined Annotation Dependent Depletion, M-CAP: Mendelian Clinically Applicable Pathogenicity, T: tolerated, D: damaging/disease causing, B: benign, N: polymorphism/neutral, P: polymorphism automatic, L: low, M: medium, PP: probably pathogenic, LB: likely benign, NA: not available. Transcript references: NM_001005742 (*GBA*), NM_198578 (*LRRK2*), NM_004562 (*PARK2*), NM_019112 (*ABCA7*), NM_003105 (*SORL1*).

Supplementary Table 4: Pathological findings in genetic variant carriers.

	Case	Sex	Age at death	Genetic variant	*APOE* ε genotype	Braak α-synuclein stage	Braak neurofibrillary tangle stage	CERAD	Thal phase
Group 1	1	M	76	*PARK2* p.A82E	34	5	3	B	1
Group 2	1	F	61	*GBA* p.D140H (p.D179H) *GBA* p.326K (p.E365K)	34	6	1	B	3
	2	M	73	*GBA* p.326K (p.E365K)	34	6	3	C	3
	3	M	67	*GBA* p.N370S (p.N409S)	33	6	2	O	0
	4	M	79	*SORL1* p.S577P	34	6	1	A	2
	5	M	84	*GBA* p.T369M (p.T408M)	34	6	2	O	3

CERAD: Consortium to Establish a Registry for Alzheimer's Disease.



Supplementary Figure 1: Graphical representation of total amount of 1st degree relatives with PD, PDD or DLB versus AD or dementia in *GBA* variant carriers (a) and *APOE* ε4 allele carriers (b). A circle represents a female patient; a square represents a male patient; a diamond represents a patient with unknown sex; black indicates patients with DLB, grey indicates patients with PD or PDD; white indicates patients with AD or unspecified dementia. DLB: dementia with Lewy bodies, PD: Parkinson's disease, PDD: Parkinson's disease dementia, AD: Alzheimer's disease.

3.2

Chapter 3.2

Neuropathological and genetic characteristics of a post-mortem series of cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease

Hanneke Geut, Leonie JM Vergouw, Yvonne Galis, Angela Ingrassia, Frank Jan de Jong, Marialuisa Quadri, Vincenzo Bonifati, Afina W. Lemstra, Annemieke J.M. Rozemuller, Wilma D.J. van de Berg

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Abstract

Introduction

The disease course of dementia with Lewy bodies (DLB) can be rapidly progressive, clinically resembling Creutzfeldt-Jakob's disease (CJD). To better understand factors contributing to this rapidly progressive disease course, we describe load and distribution of neuropathology, and the presence of possible disease-associated genetic defects in a post-mortem series of DLB cases clinically suspected of CJD.

Methods

We included pathologically confirmed DLB cases with a disease duration of 3.5 years or less from the Dutch Surveillance Center for Prion Diseases, collected between 1998 and 2014. Lewy body disease (LBD) and Alzheimer's disease (AD)-related pathology were staged and semi-quantitatively scored in selected brain regions. Whole exome sequencing analysis of known disease-associated genes, copy number analysis, *APOE* ε genotyping and *C9orf72* repeat expansion analysis were performed to identify defects in genes with a well-established involvement in Parkinson's disease or AD.

Results

Diffuse LBD was present in nine cases, transitional LBD in six cases and brainstem-predominant LBD in one case. Neocortical alpha-synuclein load was significantly higher in cases with intermediate-to-high than in cases with low-to-none AD-related pathology (p=0.007). We found two *GBA* variants (p.D140H and p.E326K) in one patient and two heterozygous rare variants of unknown significance in *SORL1* in two patients.

Conclusion

A high load of neocortical alpha-synuclein pathology was present in most, but not all DLB cases. Additional burden from presence of concomitant pathologies, synergistic effects and specific genetic defects in the known disease-associated genes may have contributed to the rapid disease progression.

Introduction

Dementia with Lewy bodies (DLB) is a neurodegenerative dementia characterized by parkinsonism, fluctuating cognitive symptoms, visual hallucinations, REM-sleep behavior disorders and neuroleptic sensitivity.[1] Clinical symptoms of DLB are gradually progressive, with a median disease duration of seven to eight years.[2] However, DLB may occasionally present with a rapid deterioration leading to death within three years from the start of the first symptoms.[3-9] These rapidly progressive DLB cases frequently show additional focal neurological signs such as myoclonus, pyramidal and cerebellar signs, and akinetic mutism.[3-9] As these neurological signs are core symptoms of Creutzfeldt-Jakob's disease (CJD), a rapidly progressive prion disease[10], the clinical differential diagnosis often includes CJD. In autopsy-series of clinically possible and probable CJD cases, DLB was the neuropathological diagnosis in 2-8% of cases.[3-5] To date, it is unknown whether these cases should be regarded as a distinct entity within the Lewy body disease (LBD) spectrum. Studying the neuropathological and genetic correlates of this clinical phenotype may aid in a better understanding of factors contributing to the rapidly progressive disease course in DLB.

The main neuropathological lesions in DLB are alpha-synuclein immunoreactive Lewy bodies (LBs) and Lewy neurites (LNs). In most DLB cases, LB pathology is present in brainstem and limbic (transitional LBD), or in brainstem, limbic and neocortical areas (diffuse LBD).[11] Additionally, Alzheimer's disease (AD)-related changes including amyloid-β plaques, neurofibrillary pathology and neuritic plaques are often present.[12] Neuropathological correlates of disease progression and survival in DLB have previously been assessed in prospectively[13] and retrospectively studied DLB[14] or combined Parkinson's disease (PD) dementia and DLB[15] cohorts. These studies revealed that patients with diffuse LBD had a shorter disease duration[13-15] and showed a more rapid cognitive decline[14] than patients with transitional LBD. Both a higher alpha-synuclein load and amyloid-β load, and to a lesser extent also neurofibrillary pathology load, were associated with a shorter disease duration.[13]

To better understand neuropathological and genetic factors contributing to a rapidly progressive disease course in DLB, we describe here the load and distribution of alpha-synuclein pathology and concomitant pathology, and the presence of possible disease-associated genetic defects in a post-mortem series of DLB cases suspected of CJD from the Dutch Surveillance Centre for Prion Diseases.

Methods

Patient selection

All cases clinically suspected of CJD with LBD at autopsy collected in the period from 1998 to 2014 by the Dutch Surveillance Center for Prion Diseases at the University Medical Center in Utrecht were included. The Dutch Surveillance Center obtained permission for

brain autopsy and written informed consent for use of clinical, pathological and genetic data for research purposes from the patients during life or from their next of kin after death. The medical ethics committee (MEC) of the University Medical Center Utrecht approved all procedures of autopsy. We retrieved information from medical records on symptoms and signs of DLB and sporadic CJD as described by the McKeith criteria and the criteria from the World Health Organization (WHO).[10,11] Symptoms were only regarded as present or not present when explicitly stated in the clinical information. An experienced neurologist (AL) classified all patients to the clinical criteria for DLB[11] and WHO criteria for CJD[10].

Inclusion criteria for this study were: 1) presence of LBD at autopsy, 2) negative screen for prion protein at autopsy, 3) disease duration of 3.5 years or less from the start of first symptoms, 4) no other major neurological or systemic diseases that provided sufficient explanation for a rapid deterioration, and 5) presence of sufficient clinical data.

Neuropathological assessment

Post-mortem examination was performed within 4 to 8 hours post-mortem. A total of 25 tissue blocks were taken from the following regions: frontal, parietal, temporal and occipital cortices, hippocampus, striatum, thalamus, mesencephalon, pons, medulla oblongata and cerebellum.

After decontamination using 98% formic acid for five minutes, brain tissue blocks were formalin-fixed, paraffin-embedded and cut into sections of 10 μm thickness. Routine histological stainings were performed with haematoxylin-eosin, Gallyas silver staining and combined Luxol fast blue-periodic acid-Schiff. All regions were examined for the presence of prion protein with the use of monoclonal antibody 3F4 (1:400, Signet labs, United States). Immunohistochemistry was performed using primary antibodies against alpha-synuclein (clone KM51; 1:500; Monosan, the Netherlands), hyperphosphorylated tau (clone AT8; 1:1000; Innogenetics, Belgium), and amyloid-β (clone 6f/3d; 1:100; Dako, United States). For staging and semi-quantitative scores of alpha-synuclein pathology, Brain Net Europe (BNE) consensus guidelines[16] and modified McKeith criteria[17] were used. LBs and LNs were scored in brainstem regions, LNs in the CA2 regions and LBs in other limbic and neocortical regions. For pathological staging of neurofibrillary tangles, amyloid-β plaques and neuritic plaques, National Institute on Aging - Alzheimer Association (NIA-AA) criteria were used.[17] Mean cerebral load of alpha-synuclein, neurofibrillary and amyloid-β pathology was calculated based on semi-quantitative load of pathology in selected regions used for pathological staging (Supplementary Methods). Presence of age-related astrogliopathy (ARTAG)[18], argyrophilic grain disease[19], atherosclerosis, ischemic or hemorrhagic lesions and small vessel disease[17] was reported, and cerebral amyloid angiopathy (CAA)[20] was classified. Spongiform changes were assessed based on presence of vacuoles in the entorhinal and temporo-occipital cortex. Neuropathological assessment was performed by an experienced neuropathologist (AR), and semi-quantitative load of pathology was scored according to consensus criteria[16,17,21] by the same assessor (HG) in all cases.

Genetic analysis

Fresh-frozen temporal cortex was available for eleven cases (Table 2). Genomic DNA was isolated from this tissue with the AllPrep DNA/RNA/miRNA Universal Kit of Qiagen. The Nimblegen SeqCap EZ Exome v.2.0 44Mb kit (Roche Nimblegen, Inc., Madison, WI) was used on a HiSeq2000 sequencer (paired-end 2x100) for the whole exome sequencing (WES). We selected non-synonymous, stop-gain or stop-loss variants in exons and variants near splice sites with a mean allele frequency of <1% in the ExAC-NFE (Exome Aggregation Consortium-Non Finnish Europeans) database and the GoNL (Genome of the Netherlands) database in genes with a well-established involvement in Parkinson's disease (PD) (*SNCA*, *Parkin*, *PINK1*, *DJ1*, *LRRK2*, *GBA*, *VPS35*, *DNAJC6*, *RAB39B*), AD (*APP*, *PSEN1*, *PSEN2*, *SORL1*, *TREM2*, *APOE*, *ABCA7*) and frontotemporal dementia (*MAPT*, *GRN*). All variants in *GBA* were selected regardless the mean allele frequency reported in public databases. Subsequently, Sanger sequencing was performed to validate selected variants and to exclude false-negative results in exons and intron-exon boundaries with low coverage (<10 reads).

Furthermore, copy dosage analysis of *SNCA*, *Parkin*, *PARK7*, *PINK1* and *APP* was done using Multiplex Ligation-dependent Probe Amplification (MLPA, MRC Holland), *APOE* ε genotyping using Taqman Allelic Discrimination and *C9orf72* repeat expansions analysis using a repeat-primed PCR assay. Further details on genetic analyses techniques are provided in the Supplementary Methods.

Statistical analyses

Mean and standard deviation were calculated for normally distributed continuous data (age at onset, age at death), median and interquartile range was used for not normally distributed data (disease duration) and ordinal data (load of different types of pathology). Correlation between cerebral alpha-synuclein, neurofibrillary and amyloid-β load was assessed with Spearman's rank correlations, and the cerebral alpha-synuclein load in a group of cases with low-to-none versus a moderate-to-high level of AD-related pathology was compared using the Mann-Whitney U test. Data were considered significant if $p<0.05$. All statistical analyses were done using IBM SPSS version 22.

Results

Neuropathological diagnoses in all cases suspected of CJD

Of 512 consecutive autopsy cases between 1998 and 2014, autopsy revealed a prion disease in 296 cases (58%), including 259 cases with sporadic CJD. Neurodegenerative diseases were the most frequent alternative diagnoses (48%), followed by immune diseases (17%), malignant diseases (10%), toxic metabolic disorders (10%), vascular diseases (9%) and other causes (6%) (Supplementary Table 1). LBD was found in 26 cases (12%), of whom sixteen cases fulfilled inclusion criteria (Supplementary Methods).

Clinical diagnosis and demographics

All sixteen cases were diagnosed during life with rapidly progressive dementia. CJD was considered the most likely clinical diagnosis or was included in the differential diagnosis in all cases by the treating neurologists, and DLB was explicitly listed in the differential diagnosis in eight cases. In retrospect, thirteen cases fulfilled clinical criteria for probable DLB and three cases for possible DLB.[11] Clinical criteria categorized one case as probable CJD, eight cases as possible CJD, and seven cases as no CJD.[10] Median disease duration was 12 months (interquartile range 6.5 to 24 months), with a mean age at onset of 77 years and a mean age at death of 78 years (SD 7.3; range 62 to 87 years). Basic demographic features, clinical symptoms, ancillary investigations and clinical diagnoses of CJD and DLB are listed in Table 1.

Distribution and load of alpha-synuclein pathology

Mild to moderate atrophy was present in 50% of cases (Table 2). Moderate to severe neuronal loss in the substantia nigra was present in all cases. Brainstem-type LBD was present in one case (Figure 1, 5a-c), transitional LBD in six cases and diffuse LBD in nine cases. Eleven cases showed Braak alpha-synuclein stage 6 (Table 2). A large proportion of the cases showed a moderate to severe load of alpha-synuclein pathology in the substantia nigra (81%), temporo-occipital cortex (75%), temporal cortex (56%), and frontal cortex (38%, Figure 2).

Presence of concomitant AD-related pathology, vascular pathology and spongiform changes

All cases showed neurofibrillary pathology, with nine cases reaching Braak neurofibrillary stage 3 or higher. One case (case 8) showed ARTAG, none of the cases showed argyrophilic grain disease. Diffuse and/or classical amyloid-β positive plaques were present in thirteen cases and neuritic plaques were present in the neocortex of nine cases (Table 2). The level of AD-related pathology was none in two cases, low in six cases, intermediate in six cases and high in two cases (case 1 and 11; Figure 1, 2d-e, Table 2).

Case 16 showed severe capillary CAA, Thal stage 3 (Figure 1, 3e). Additionally, CAA type 2 was present in six cases. Signs of small vessel disease were present in three cases, and severe atherosclerosis of large vessels combined with multiple cortical and subcortical infarctions were present in two cases (case 13 and 15). Mild to severe spongiform cortical changes were present in twelve cases (75%) (Table 2).

Table 1: Basic demographic features, clinical symptoms, ancillary investigations and diagnoses of dementia with Lewy bodies and Creutzfeldt-Jakob's disease in sixteen cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease.

Sex	9 M / 7 F
Disease duration, months	12 (4–42)
Age at onset, years	77 (7.3)
Age at death, years	78 (7.3)
Presence of clinical symptoms (yes / no / NA)	
- Dementia	16 (100%)/ 0 / 0
- Parkinsonism	14 (88%)/ 2 / 0
- Fluctuating cognition	11 (69%)/ 1 / 4
- Visual hallucinations	12 (75%)/ 0 / 4
- Delusions	9 (56%)/ 0 / 7
- Neuroleptic sensitivity	9 (56%)/ 1 / 6
- History of depression	5 (31%)/ 0 / 11
- Autonomic dysfunction	3 (19%)/ 0 / 13
- Myoclonus	10 (63%)/ 1 / 5
- Pyramidal symptoms	6 (38%)/ 5 / 5
- Cerebellar symptoms	4 (25%) / 8 / 4
- Akinetic mutism	3 (19%)/ 4 / 9
Ancillary investigations	
- PSWCs on EEG	Present in 0 of 11 cases with a reported EEG (non-periodic bi- or triphasic complexes in 3 cases)
- Hyperintensities on MR-DWI	Present in 0 of 7 cases with a reported MRI
- 14-3-3 protein in CSF	2 negative; 1 inconclusive; 2 positive of 5 cases with a reported 14-3-3 CSF test
Clinical diagnosis of DLB[11]	0 no; 3 possible; 13 probable
Clinical diagnosis of CJD[10]	7 no; 8 possible; 1 probable

Data are presented as mean (SD), median (range) or n (%). PSWCs: periodic sharp wave complexes, EEG: electroencephalogram, MR-DWI: magnetic resonance diffusion weighted imaging, CSF: cerebrospinal fluid, NA: not available.

Table 2: Staging of Lewy body disease and Alzheimer's disease related pathology, presence of vascular pathology and spongiform changes, genetic variants in disease-associated genes and *APOE* ε genotype in sixteen cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease.

Case number	Sex	Age at death	Clinical criteria for DLB	Clinical criteria for CJD	Atrophy	Athero-sclerosis	Infarctions	Braak alpha-synu-clein stage	McKeith Lewy body stage	Braak neurofi-brillary stage	Thal phase for amy-loid-beta	CERAD age-re-lated score	Level of AD-related pathology	Small vessel disease	Cerebral amyloid angiopa-thy	Spon-giform changes	Genetic variants in disease-as-sociated genes	*APOE* ε genotype
1	M	74	Probable DLB	No CJD	no	no	no	6	diffuse	5	4	C	high	no	type 2 stage 2	moderate	ND	ND
2	M	79	Probable DLB	Possible CJD	moderate (global, wide ventricles)	no	no	5	transiti-onal	3	1	O	low	yes	no	mild	*SORL1* (p.R1799Q)	2/3
3	F	87	Probable DLB	Possible CJD	no	no	no	6	diffuse	2	3	O	low	no	type 2 stage 2	severe	-	3/3
4	F	81	Probable DLB	No CJD	no	no	no	6	transiti-onal	2	2	O	low	no	no	moderate	ND	ND
5	M	67	Probable DLB	No CJD	no	mild	no	6	diffuse	3	3	B	intermediate	no	type 2 stage 1	none	ND	ND
6	F	86	Probable DLB	Possible CJD	mild (frontal)	moderate	no	6	diffuse	4	3	B	intermediate	yes	no	mild	-	3/3
7	M	75	Probable DLB	No CJD	no	no	no	6	transiti-onal	2	2	O	low	no	no	mild	*GBA* (p.D140H + p.E326K)	3/3
8	M	71	Possible DLB	Possible CJD	mild (frontotemporal)	moderate	no	6	diffuse	3	3	B	intermediate	no	type 2 stage 1	mild	-	3/3
9	M	85	Probable DLB	Possible CJD	no	moderate	no	5	transiti-onal	1	3	B	low	no	no	none	-	3/3
10	F	76	Possible DLB	No CJD	mild (frontotemporal)	moderate	no	3	brainstem	1	0	O	none	no	no	none	-	2/3
11	M	75	Probable DLB	Possible CJD	mild (frontotemporal)	mild	no	6	diffuse	6	4	C	high	no	no	severe	ND	ND
12	F	80	Probable DLB	Possible CJD	no	mild	no	5	transiti-onal	1	0	O	low	no	no	mild	-	2/4
13	M	84	Probable DLB	No CJD	moderate (frontal)	severe	microscopical infarctions: CA1/CA2 region hippocampus right, caudate nucleus right and left, occipital cortex	6	diffuse	3	4	C	intermediate	yes	type 2 stage 1	severe	*SORL1* (p.D140N)	3/4
14	F	62	Possible DLB	Possible CJD	mild (frontal, amygdala, brainstem)	mild	no	4	transiti-onal	1	0	O	none	no	no	none	-	3/3
15	F	87	Probable DLB	Probable CJD	mild	severe	cerebellar left (2cm), microscopical infarctions in temporal cortex and thalamus	6	diffuse	3	5	C	intermediate	no	type 2 stage 1	mild	-	3/4
16	M	78	Probable DLB	No CJD	no	mild	no	6	diffuse	3	4	B	intermediate	no	type 1	x	x	x

CERAD: Consortium to Establish a Registry for Alzheimer's Disease, ND: not done.

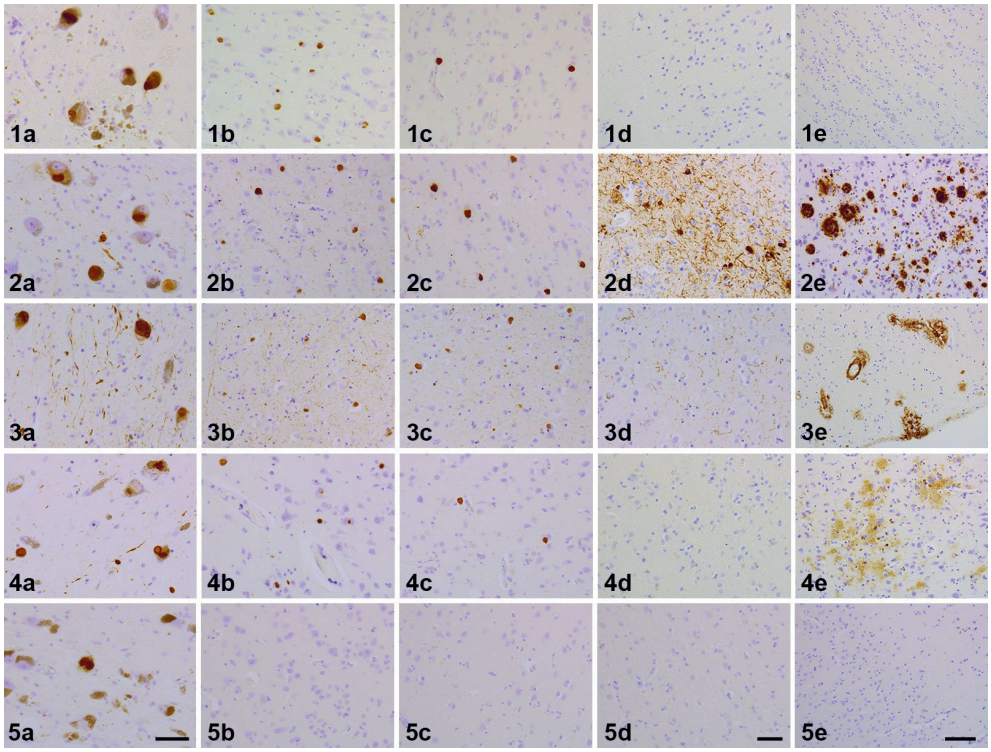

Figure 1: Representative overview of the neuropathological features visualized with immunohistochemistry in sixteen cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease.
Load of alpha-synuclein pathology was high in limbic and neocortical regions in most cases (**1b-4b, 1c-4c**), but a few cases only showed brainstem-predominant (**5a-c**) or transitional Lewy body disease (LBD). Neurofibrillary and amyloid-β pathology were absent or low in some (**1d-e, 4d-e, 5d-e**), but severe in other cases (**2d-e**). One case showed severe capillary cerebral amyloid angiopathy (**3e**). A case with two *GBA* variants (p.D140H and p.E326K) showed diffuse LBD (**4a-c**) and low levels of Alzheimer's disease (AD)-related pathology (**4d-e**). One case showed nigral degeneration and brainstem-predominant LBD (5a-c) without signs of AD-related pathology (**5d-e**).
Representative microscopy images from case 4 (**1a-e**), 11 (**2a-e**), 16 (**3a-e**), 7 (**4a-e**) and 10 (**5a-e**). Immunohistochemistry against alpha-synuclein (clone KM51) was performed on **a:** substantia nigra, **b:** trans-entorhinal cortex and **c:** temporal cortex. **d:** Immunohistochemistry against hyperphosphorylated tau (clone AT8) on temporal cortex. **e:** Immunohistochemistry against amyloid-β (clone 6f/3d) on temporal (1e, 2e, 4e and 5e) and frontal (3e) cortex. The scale bar in 5a represents 50 µm and applies to 1a-5a, 1b-5b and 1c-5c. The scale bar in 5d represents 50 µm and applies to 1d-5d. The scale bar in 5e represents 100 µm and applies to 1e-5e.

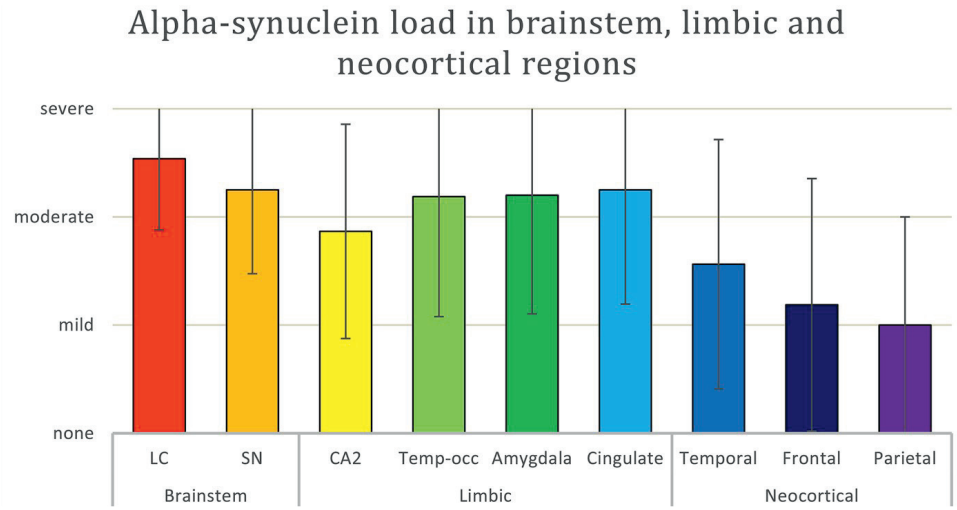

Figure 2: Semi-quantitative load of alpha-synuclein pathology in brainstem, limbic and neocortical brain regions in sixteen cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease.
Lewy bodies (LBs) and Lewy neurites (LNs) were scored in brainstem regions, LNs in the CA2 region and LBs in other limbic and neocortical regions. Bars represent mean values, error bars represent standard deviations. LC: locus coeruleus, SN: substantia nigra, CA2: cornu ammonis region 2, temp-occ: temporo-occipital cortex, cingulate: posterior cingulate gyrus, temporal: medial temporal gyrus, frontal: frontal gyrus (Brodmann area 10), parietal: inferior parietal lobe.

Correlations between cerebral load of alpha-synuclein, neurofibrillary and amyloid-β pathology

Cases with an intermediate-to-high load of AD-related pathology (n=8) had a significantly higher cerebral load of alpha-synuclein pathology compared to cases with no-to-low level of AD-related pathology (n=8, p=0.007). The load of alpha-synuclein pathology was significantly correlated to both the load of neurofibrillary (Spearman's rho 0.757; p=0.001) and amyloid-β pathology (Spearman's rho 0.707; p=0.002).

Presence of genetic defects and their neuropathological correlates

In three of the eleven cases (45%) analyzed, possible disease-associated variants were detected in genes with a well-established involvement in PD or AD (Supplementary Table 2).

In case 7, a rare variant and a common variant were detected in *GBA* (p.D140H and p.E326K) and confirmed by Sanger sequencing. E326K and E326K+D140H variants have been associated with a more rapid cognitive decline in PD.[22,23] At autopsy, case 7 showed a high load of neocortical alpha-synuclein and a low level of AD-related pathology (Figure 1, 4a-e).

Additionally, the p.R1799Q and p.D140N variants in *SORL1* were found in case 2 and 13 respectively, and confirmed by Sanger sequencing. These variants have an allele frequency

of 0.005% and 0.03% in the GnomAD (Genome Aggregation Database) respectively. The p.D140N variant is predicted to be pathogenic by half of the *in-silico* programs, whilst the p.R1799Q variant is predicted to be likely benign by the majority of programs. The case with the p.D140N variant showed an intermediate level of AD-related pathology, whereas the case with the likely benign p.R1799Q variant showed only a low level of AD-type pathology.

No *SNCA*, *Parkin*, *PARK7*, *PINK1* or *APP* dosage abnormalities and no pathogenic *C9orf72* repeat expansions were found. Three cases carried an *APOE* ε4 allele, of which one had no AD-related pathology and two had an intermediate level of AD-related pathology (Table 2).

Discussion

Until now, few studies have reported the neuropathological characteristics of DLB cases suspected of CJD. Here, we describe neuropathological and genetic features in sixteen of these cases collected by the Dutch Surveillance Center for Prion Diseases. A high load of neocortical alpha-synuclein pathology was present in most, but not all DLB cases, and cases showed a variable load of AD-related pathology. We found two *GBA* variants in one patient and two heterozygous rare variants of unknown significance in *SORL1* in two patients.

In the current study, ten cases retrospectively fulfilled clinical criteria for possible or probable CJD, and all cases retrospectively fulfilled clinical criteria for possible or probable DLB, with a median disease duration of 12 months. The overlap of clinical symptoms in cases with rapidly progressive dementias is well-known, and it has been proven difficult to discriminate rapidly progressive DLB and CJD based on clinical symptoms and signs.[3-5,7]

The neuropathological correlates of rapidly progressive DLB have only been studied in few case-studies and very small case-series. Transitional LBD was present in four cases, and diffuse LBD in 16 out of 20 cases described.[4,7,8,24] The level of AD-related pathology according to the NIA-AA[17] ranged from none to severe.[7-9] Additionally, concomitant pathology included argyrophilic grain disease[7], TDP-43 positive inclusions[8], atherosclerosis[8], infarctions[8], small vessel disease[7-9], CAA[7], and a subarachnoidal hemorrhage[8]. In conclusion, the neuropathology described in these cases was variable, but studies were small with heterogeneous inclusion criteria.

In 56% of DLB cases suspected of CJD in the current study, the neuropathology was characterized by diffuse LBD, with an intermediate to high load of alpha-synuclein pathology in neocortical regions. In comparison, a previous large post-mortem series of 807 DLB cases (mean disease duration 8.8 ± 4.0 years) revealed diffuse LBD in 57% of the cases.[14] However, due to differences in case selection, a direct comparison of different cohorts should be interpreted with caution.[25] Although diffuse LBD has been related to a more rapidly progressive disease course in DLB[13,14] and LBD[15], our results show that the pathological correlate of rapidly progressive DLB can be transitional LBD or even brainstem-predominant LBD.

The load of AD-related pathology varied from none to severe in this study. A negative correlation between load of AD-related pathology and survival in DLB has been shown by neuropathological studies[13,15] and a recent cerebrospinal fluid biomarker study[26]. However, the current study shows that DLB symptoms can also progress rapidly in cases with little concomitant pathology. Also, concomitant vascular pathology was present in several cases, which may have added to disease progression.

Spongiform changes were found in 75% of cases in the current study, which is in line with a previous post-mortem study of 40 DLB cases, where 82% showed spongiform changes.[27] In this study, there was no correlation between spongiform changes and disease duration[27], which is corroborated by the low frequency of severe spongiform changes in the current series. The heterogeneous neuropathology in our cases is in line with previous reports on the presence of neuropathological hallmarks in DLB cases suspected of CJD[7-9], and with the neuropathology of DLB cases in general[13,14].

In this study, a higher alpha-synuclein load was associated with a higher load of AD-related pathology. This is in line with results from previous autopsy studies, that revealed a correlation between alpha-synuclein pathology and AD-related pathology in LBD[15], and DLB[13] cases. Together with evidence from *in vitro* and animal cross-seeding experiments, this suggests synergistic interactions between hyperphosphorylated tau, amyloid-β and alpha-synuclein aggregates.[28]

The relation between genetic defects and a rapidly progressive phenotype in DLB has been understudied. In a few rapidly progressive DLB cases, a genetic screening has been performed, without finding evidence for presence of genetic defects.[8,24]

Interestingly, in one diffuse LBD case with a low level of AD-related pathology, a combination of two genetic variants was found in *GBA* (p.D140H and p.E326K). This combination of variants has been described before in Gaucher disease[23] and PD[22]. PD carriers of the p.E326K variant and the D140H+E326K complex allele were shown to have a faster cognitive decline than non-*GBA* associated PD cases.[22] As *GBA* variants have been shown to be associated with more severe motor and cognitive dysfunction in DLB[29], these variants may have contributed to the rapid cognitive decline.

Furthermore, two other heterozygous variants in the *SORL1* gene (p.D140N and p.R1799Q) were observed in two different cases. According to recent criteria for *SORL1* variants in AD, both variants are categorized as 'likely benign'.[30] However, as these criteria have been adopted for AD, the role of these variants in DLB disease progression is still uncertain.

Limitations of the current study are the small sample size, limited availability of tissue for genetic analysis, inclusion of cases based on clinical referrals, and the retrospective nature of the clinical data. Additionally, rapid clinical deterioration in DLB can be induced by a hypersensitive reaction to neuroleptic treatment, which may be misinterpreted as signs of CJD.[6] This iatrogenic cause of disease progression may play a role in some cases in this study. Studies in larger, prospectively followed cohorts of patients are needed to draw more

generalizable conclusions on the neuropathological or genetic substrates of rapid disease progression in DLB, especially regarding the clinical and pathological heterogeneity in this group of patients. However, this may be difficult to realize in clinical practice, as DLB cases clinically suspected of CJD are very rare.

In short, a high load and neocortical distribution of alpha-synuclein pathology is present in some, but not all DLB cases in this post-mortem series. This suggests that a different set of factors contribute to the rapidly progressive disease course in cases with no or a low load of neocortical alpha-synuclein pathology. Additional burden from presence of concomitant pathologies, synergistic effects and specific genetic defects may have contributed to the disease progression in some cases. Understanding which factors contribute to a rapid disease progression in DLB could aid in the search for biomarkers that enable clinicians and researchers to select patients for therapeutic strategies.

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Supplementary Information

Case selection

Of 26 cases with LBD without presence of prion protein, collected from 1998 to 2014, 16 were included in the study whereas the remaining 10 cases did not meet the inclusion criteria. A disease duration of four years or longer was present in five cases, other neurological diseases were apparent in three cases (one case with an intracerebral B-cel lymphoma, one case with a large recent infarction, and one case with microcephaly), and sepsis with multiple organ failure or bronchopneumonia coincided with the neurological decline in two cases. Thus, sixteen cases were included in the current study.

Calculation of mean load of neurofibrillary, amyloid-β and alpha-synuclein pathology

Alpha-synuclein pathology was calculated as the mean of temporo-occipital, cingulate, temporal, frontal and parietal scores. Neocortical load of neurofibrillary pathology was calculated as the mean of the entorhinal, temporo-occipital, temporal, occipital-peristriatal and occipital-striatal scores, and neocortical amyloid-β load was calculated as the mean of entorhinal, temporo-occipital, cingulate, temporal, frontal, parietal and occipital scores.

DNA quality assessement

All samples were snap frozen in liquid nitrogen at autopsy and stored at -80°C until use. Degradation of DNA was assessed using DNA electrophoresis on agarose gels, which did not reveal DNA degradation in any of the samples. Quantification of DNA purity by Nanodrop® showed 260/280 ratios above 1.8 for all samples.

Whole exome sequencing

The Burrows-Wheeler alignment tool was used to align reads to the human reference genome (hg19). Picard (http://broadinstitute.github.io/picard) and the Genome Analysis Toolkit (GATK) were used to process aligned reads following standard procedures.[1] We determined single nucleotide variations using GATKs HaplotypeCaller, and used ANNOVAR for functional annotation.[2]

Sanger sequencing

Direct sequencing of both strands was done using the Big Dye Terminator chemistry version 3.1 (Applied Biosystems), and fragments were run on an ABI3130 or ABI3730 sequencer. SeqScape (version 2.6) was used for analysis.

Multiplex Ligation-dependent Probe Amplification

The P051-D1 Parkinson and P170-C2 APP MLPA kits (MRC Holland) were used according to the manufacturer's protocol. An ABI3130 or ABI3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA) and GeneMarker Ver.2.4.0 (SoftGenetics, State College, PA, USA) were used for further analysis.

APOE ε genotyping

APOE ε genotyping (rs7412 and rs429358) was done using Taqman Allelic Discrimination. Signals were read with the Taqman 7900HT (Applied Biosystems Inc.) and analyzed using sequence detection system 2.3 software (Applied Biosystems Inc.).

C9orf72 repeat expansions analysis

To detect pathogenic *C9orf72* repeat expansions, a previously described repeat-primed PCR assay[3] was used. An ABI3130 or ABI3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA) and GeneMarker Ver.2.4.0 (SoftGenetics, State College, PA, USA) were used for further analysis. A pathogenic *C9orf72* expansion was defined as more than 30 repeats.[3]

Supplementary Table 1: Neuropathological diagnosis in 512 consecutive autopsy cases in period 1998-2014 at the Dutch Surveillance Center for Prion Diseases.

Prion disease	**296**		
		Sporadic CJD	259
		Sporadic CJD	252
		Sporadic CJD + AD	7
		Other prion diseases	37
Non-prion disease	**216**		
		Neurodegenerative diseases	104
		AD	46
		LBD	26
		Mixed AD + vascular pathology	14
		PSP	4
		FTD-tau or FTD-TDP	8
		ALS	2
		Multiple sclerosis	4
		Immune diseases	37
		Limbic encephalitis	27
		Viral infections	4
		Neurocoeliac disease	4
		HIV encephalopathy	2
		Malignant diseases	22
		Myeloproliferative disease	11
		Metastases	5
		Intravascular lymphoma	3
		Astrocytoma or glioblastoma	3
		Toxic metabolic disorders	21
		Vascular diseases	20
		Thrombotic or vascular lesions	18
		Non-CAA angiopathy	2
		Other	12
Total	**512**		

CJD: Creutzfeldt-Jakob's disease, AD: Alzheimer's disease, LBD: Lewy body disease, PSP: progressive supranuclear palsy, FTD: frontotemporal dementia, ALS: amyotrophic lateral sclerosis, HIV: human immunodeficiency virus, CAA: cerebral amyloid angiopathy.

Supplementary Table 2: Variants in disease-associated genes in dementia with Lewy bodies cases clinically suspected of Creutzfeldt-Jakob's disease.

Case nr	Gene	Position	db SNP 142 accession number	Nucleotide change	Amino acid change	Mean allele frequency GoNL (%)	Mean allele frequency ExAC-NFE (%)	Mean allele frequency GnomAD (%)	Functional predic-tions: pathogenic (total)	Pathogenicity in AD according to criteria by Holstege et al.[4]	Pathogenic in literature
7	*GBA*	Exon 6	rs147138516	c.535G>C	p.D140H (p.D179H)	0.1	0.01	0.01	4 (10)	-	Yes[5]
7	*GBA*	Exon 9	rs2230288	c.1093G>A	p.E326K (p.E365K)	2.30	1.20	1.01	3 (9)	-	Risk factor[5]
2	*SORL1*	Exon 40	rs530863434	c.5396G>A	p.R1799Q	-	0.01	0.005	1 (10)	Likely benign	Not described
13	*SORL1*	Exon 3	rs140888526	c.418G>A	p.D140N	-	0.05	0.03	5 (10)	Likely benign	Not likely[6]

ExAC-NFE: Exome Aggregation Consortium - Non Finnish Europeans, GoNL: Genome of the Netherlands, GnomAD: Genome Aggregation Database (non-Finnish Europeans). Transcript references: NM_001005742 (*GBA*), NM_198578 (*LRRK2*) and NM_004562 (*Parkin*).

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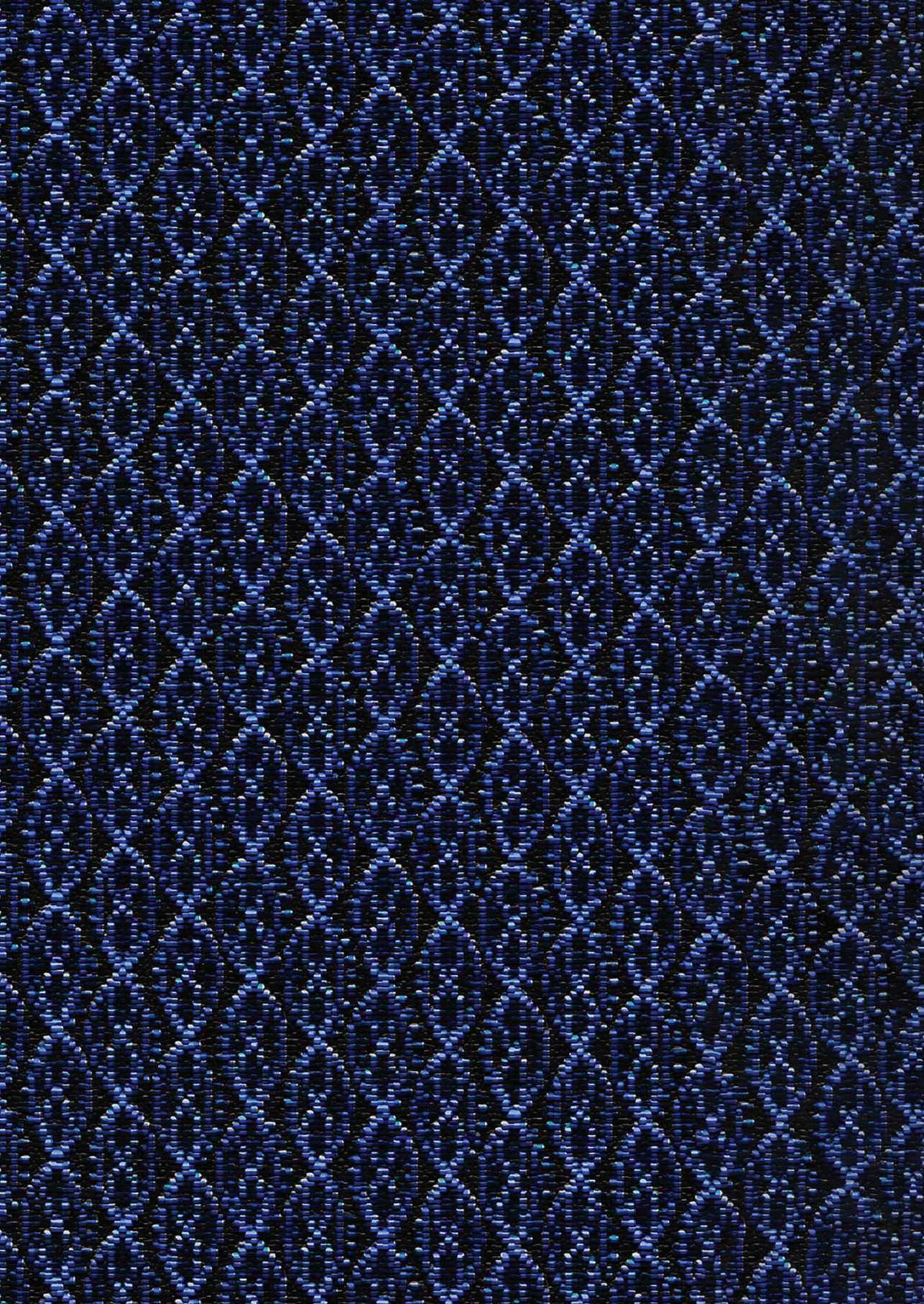

Part 4

LRP10 variants associated with dementia with Lewy bodies and related disorders

4.1

Chapter 4.1

LRP10 variants in Parkinson's disease and dementia with Lewy bodies in the South-West of the Netherlands

Leonie J.M. Vergouw*, Annemieke Ruitenberg*, Tsz Hang Wong*, Shamiram Melhem, Guido J. Breedveld, Chiara Criscuolo, Giuseppe De Michele, Frank Jan de Jong, Vincenzo Bonifati, John C. van Swieten, Marialuisa Quadri

* Shared first author

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Abstract

Objective

To analyse *LRP10* variants, recently associated with the development of Parkinson's disease (PD), Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB), in a series of patients and controls from the South-West of the Netherlands (Walcheren).

Methods

A series of 130 patients with PD, PDD or DLB were clinically examined, and a structured questionnaire used to collect information about family history of PD and dementia. The entire *LRP10* coding region was sequenced by Sanger methods in all patients, and haplotype analysis was performed for one recurrent *LRP10* variant. The fragments containing possibly pathogenic *LRP10* variants were sequenced in 62 unaffected control subjects from the same region. Other known PD-associated genes were analyzed by exome sequencing and gene dosage in the carriers of *LRP10* variants.

Results

Four patients were carriers of a rare heterozygous, possibly pathogenic *LRP10* variant: p.Arg151Cys, p.Arg263His, and p.Tyr307Asn. None of these variants was detected among the controls, nor were additional mutations identified in known PD-associated genes in the four *LRP10* variant carriers. The previously reported p.Tyr307Asn variant was identified in two patients (with PD and PDD), who are connected genealogically within six generations, and in one of their relatives with cognitive decline. Haplotype analysis suggests a common founder for the p.Tyr307Asn variant carriers analyzed.

Discussion

We report three possibly pathogenic *LRP10* variants in patients with PD and PDD from a local Dutch population. The identification of additional patients carrying the p.Tyr307Asn variant provides some further evidence that this variant is pathogenic for PD and PDD.

Introduction

Parkinson's disease (PD), Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB) are common neurodegenerative diseases, which share clinical, pathological and genetic features.[1,2] These disorders occur in approximately 1-2% of the population above 60 years old[3,4], and their common hallmark is Lewy pathology, observed primarily in the brainstem in PD, and more diffusely throughout the brain in PDD and DLB[2,5].

Although PD, PDD and DLB manifest mostly as sporadic diseases, during the last decades mounting evidence showed that genetic factors play an important role in the disease etiopathogenesis.[6-8] Recently, genetic defects in the *low-density lipoprotein receptor related protein 10* gene (*LRP10*) have been reported in familial PD, PDD and DLB.[9]

The aim of this study was to screen *LRP10* in a series of patients and unaffected subjects from an isolated region in the South-West of the Netherlands.

Methods

Participants

Between 2007 and 2010 we ascertained a series of 130 patients with PD (n=71), PDD (n=55), or DLB (n=4), as well as 62 unrelated and unaffected subjects originating from a region in the South-West of the Netherlands (Walcheren). This area had maintained the features of an island until 1870, and for centuries its population remained geographically isolated from the surrounding areas. All patients were neurologically examined by a neurologist (AR). Structured questionnaires were used to collect information about family history of PD and dementia and The Mini Mental State Examination (MMSE) was administered as screening tool for the cognitive status. Clinical follow-up was available until July 2018. The diagnosis of PD required the exclusion of secondary causes of parkinsonism, and the presence of at least two of the following signs: bradykinesia, rigidity, or rest tremor; or, presence of one of these signs together with improvement with dopaminergic medications. Diagnosis of DLB or PDD was made according to the criteria described by McKeith and co-workers[10] and by Emre and co-workers[11], respectively. Unaffected spouses of patients or of relatives were recruited as controls. Blood samples were collected from patients, available relatives and controls for DNA isolation. The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam, the Netherlands (MEC-2005-206) and written informed consent was obtained by all participating subjects.

Genetic Studies

The entire open reading frame and the intron-exon boundaries of *LRP10* were sequenced in all patients using a reported Sanger protocol[9] with minor modifications (Supplementary Information). Variants were considered as possibly pathogenic if they were: (1) present in

heterozygous state (as expected for variants acting in a dominant fashion); (2) rare, defined as with a minor allele frequency (MAF) <0.001 by the Genome Aggregation Database (GnomAD v2.1.1; https://gnomad.broadinstitute.org/); (3) exonic and non-synonymous, or predicted to affect splicing; and (4) predicted as pathogenic by at least five of 11 *in-silico* programs (Supplementary Information). The *LRP10* fragments containing possibly pathogenic variants identified in the patients were sequenced in the controls.

Whole exome sequencing (WES) and multiple ligation probe amplification (MLPA, P051-Parkinson mix 1, MRC Holland) were also performed in the patients who carried possibly pathogenic *LRP10* variants to rule out mutations in other known genes associated with PD or parkinsonism (Supplementary Table 1).

Haplotype analysis of a 6-Mb genomic region flanking *LRP10* was performed in the carriers of one recurrent *LRP10* variant (c.919T>A, p.Tyr307Asn), by typing short tandem repeat (STR) markers. DNA of the Italian PDD patient previously reported by us with the same variant[9], was also included in this analysis (Supplementary Figure 1).

LRP10 protein conservation analysis was performed using the T-Coffee multiple sequence alignment program (https://www.ebi.ac.uk/Tools/msa/tcoffee/) (Supplementary Figure 2). Details on the methodologies are reported in the Supplementary Information.

Results

The demographic and clinical characteristics of the 130 index patients are reported in Supplementary Table 2. The index patients had a mean age at disease onset of 62.4 ± 9.6 years and the 62 unrelated and unaffected participants had a mean age of 63.5 ± 12.3 years at recruitment.

Four index patients carried *LRP10* variants that fulfill our criteria for being considered as possibly pathogenic: c.451C>T/p.Arg151Cys (GnomAD MAF 0.000056, 16 alleles) in one patient; c.788G>A/p.Arg263His (GnomAD MAF 0.000007, 2 alleles) in another patient; and c.919T>A/p.Tyr307Asn (GnomAD MAF 0.000059, 15 alleles) in two other patients (Table 1). None of these variants were detected among the 62 unaffected subjects. Additional variants detected in patients but not fulfilling our criteria to be considered as possibly pathogenic are reported in Supplementary Table 3. Our WES and MLPA analyses in the four patients carrying *LRP10* possibly pathogenic variants detected no pathogenic variants in any of the other known genes associated with PD or parkinsonism (WES reached an average depth >190x, with 99.1% of the target region covered >20x).

Two patients, whose genealogy can be traced back to a common ancestor within six generations, carry the *LRP10* p.Tyr307Asn heterozygous variant. The diagnosis of PD in the first patient (Family 1, II-2) was established based on rigidity, bradykinesia, reduced arm swing and reduced facial expression, after he presented with rest tremor of the left hand at 63 years of age. The patient had multiple depressive episodes. He did not report PD or dementia among his first-degree relatives (Figure 1). DNA was available from two of his

Table 1: Details of the possibly pathogenic *LRP10* variants identified in this study and previous studies.

Genomic position	Nucleotide change	Amino acid change	Exon	Coding effect	dbSNP accession number	MAF GnomAD (alleles)	Functional predictions: pathogenic (total)	Study	Patients	Diagnosis	AAO (years)	AAD (years)	First symptom
14:23345076	c.919T>A	p.Tyr307Asn	5	missense	rs139650807	0.005% (15)	11/11	Current	Patient II-2 (Family 1)	PD	63	-	Rest tremor
								Current	Patient II-2 (Family 1)	PDD	69	79	Motor difficulties
								Current	Patient II-3 (Family 1)	Cognitive decline*	78	-	NA
								Quadri et al., 2018[9]	Patient II-1 (Family 5)	PDD	71	-	Rest tremor
								Tesson et al., 2018[12]	Patient I-9 (FPD-083)	PD	73	NA	Rest tremor
								Tesson et al., 2018[12]	Patient II-1 (FPD-083)	PD	45	NA	Rest tremor
14: 23344945	c.788G>A	p.Arg263His	5	missense	rs372858291	0.0007% (2)	6/11	Current	Patient III-1 (Family 3)	PD	68	-	Rest tremor and bradykinesia
14:23344608	c.451C>T	p.Arg151Cys	5	missense	rs774043484	0.006% (16)	9/11	Current	Patient II-2 (Family 4)	PDD	66	74	Rest tremor and hypokinesia

GRCh37: genome reference consortium human 37; *LRP10* variant nomenclature is assigned based on reference sequence NM_014045.4. MAF: minor allele frequency, GnomAD: Genome Aggregation Database, AAO: age at onset, AAD: age at death, *: progressive cognitive decline of neurodegenerative nature, NA: not available, PD: Parkinson's disease, PDD: Parkinson's disease dementia.

offspring, asymptomatic at the age of 33 and 47 years, respectively; both also carried the *LRP10* p.Tyr307Asn variant in heterozygous state (Figure 1 and Supplementary Figure 1). The second patient carrying the *LRP10* p.Tyr307Asn variant (Family 2, II-2) developed motor difficulties in the right leg at the age of 69. PD was diagnosed one year later based on bradykinesia, reduced facial expression, hypersalivation and orthostasis, in absence of rest tremor. He developed a paresis of the left arm after a small intracerebral hemorrhage in the right basal ganglia at the age of 75, followed by cognitive deterioration and periods of confusion. A dementia was diagnosed one year later and he died at 79 years old. He did not report PD among his first-degree relatives. However, his brother was recently diagnosed with a progressive cognitive decline of neurodegenerative nature, and DNA testing revealed that he also carries the *LRP10* p.Tyr307Asn variant (Figure 1 and Supplementary Figure 1).

A third patient (Family 3, III-1), carrying a *LRP10* p.Arg263His variant, developed a rest tremor of both hands and bradykinesia at 68 years of age. The PD diagnosis was established two years later, when rigidity and reduced facial expression were also present. He has mild memory impairments, concentration and orientation problems at the current age of 78. One of his cousins was also diagnosed with PD (paternal side), the patient's father had memory complaints and his three sisters were affected by dementia (Figure 1). Unfortunately, co-segregation studies could not be performed in the affected relatives due to unavailability of DNA.

A fourth patient (Family 4, II-2), carrying a *LRP10* p.Arg151Cys variant, was diagnosed with PD based on hypokinesia and rest tremor of the left hand at 66 years of age. He developed rigidity, hallucinations and depression several years later, and memory complaints at the age of 70. A diagnosis of dementia was established at 73 years of age, one year before he died. His father suffered from Alzheimer's disease, walking problems and frequent falls which started at the end of his seventies.

As expected from the genealogical links, our haplotype studies showed that the patients in the two Dutch families with the *LRP10* p.Tyr307Asn variant share an extended haplotype of several megabases flanking the *LRP10* gene. The Italian PDD patient previously reported by us with the same variant [9] shares a smaller haplotype of ~1Mb, therefore compatible with a common but more distant ancestor.

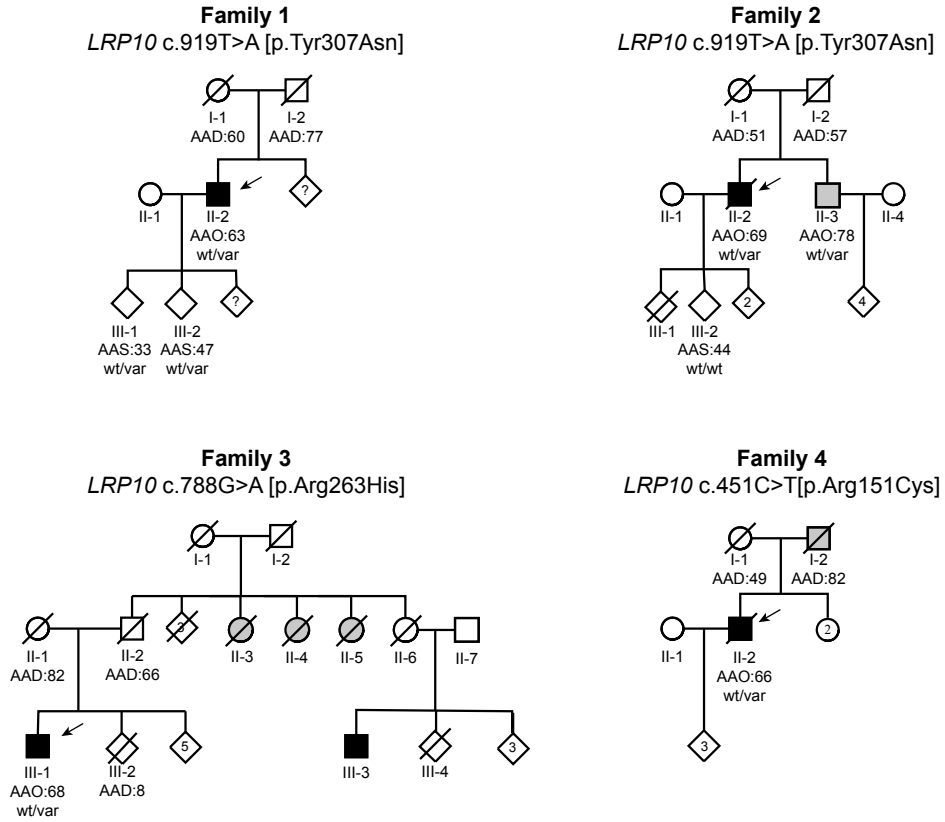

Figure 1: Pedigrees of patients carrying *LRP10* variants.
Circles indicate females, squares indicate males, diamonds indicate sex-disguised individuals; black symbols indicate patients affected by Parkinson's disease or Parkinson's disease dementia; grey symbols indicate patients with Alzheimer's disease, cognitive decline or unspecified type of dementia; diagonal lines indicate deceased individuals; arrows indicate index patients; numbers within a circle, square or diamond indicate the number of individuals; question marks indicate that there is no information about individuals' disease status. AAD: age at death, AAO: age at onset, AAS: age at sampling, wt: wild-type, var: variant.

Discussion

Here, we report three rare, possibly pathogenic *LRP10* variants in a relatively small series of 130 patients from the South-West of the Netherlands. The identified variants were absent in 62 unaffected subjects from the same region, and extremely rare in public databases (Table 1). Moreover, WES and MLPA performed in our four index patients with *LRP10* variants detected no pathogenic variants in any of the other genes previously associated with PD or parkinsonism.

Of interest, the p.Tyr307Asn variant identified here in two Dutch patients with PD and PDD and in one relative with cognitive decline, was initially observed by us in one patient with PDD from Italy [9]. The same variant was subsequently reported in a parent-offspring pair with PD, in a screening of 25 PD/DLB French families[12]; another two relatives affected by PD in that family did not share the p.Tyr307Asn variant, and might represent phenocopies.

Thus, the *LRP10* p.Tyr307Asn variant has been identified at least three times so far, in independent studies targeting patients of European ancestry with familial forms of PD, PDD or DLB, and including a total of 701 unrelated probands: 608 patients of European ancestry in our initial study[9]; 25 French patients studied by Tesson and colleagues[12]; and 68 Dutch patients in the current study (here we consider Family 1 an 2 as part of one extended kindred). The frequency of this variant among patients with familial forms of PD, PDD or DLB (3/701) is significantly higher compared to that present in GnomAD v2.1.1 (13/74109, only considering non-Finnish Europeans and Latino individuals, two-sided Fisher's Exact test, p=0.0048). Furthermore, functional studies showed that this variant leads to decreased stability of the LRP10 protein compared to the wild-type[9]. Taken together, these genetic and functional data support the contention that this variant plays a role in the development of PD and PDD. The penetrance of the p.Tyr307Asn variant as well as of the p.Arg151Cys and p.Arg263His variants might be incomplete. However, an accurate estimation is currently impossible because, although PD occurred sporadically in some carriers, in many of them the parents had died before the age at disease development observed in their offspring, and the patients' offspring are still younger than that age.

Considering together all patients with p.Tyr307Asn reported so far (n=6), the initial diagnosis was PD in five patients and progressive cognitive decline of neurodegenerative nature in one, rest tremor was the presenting sign in four out of the five patients diagnosed with PD, and mean age at onset was 66.5 years (SD:11.6; range:45-78; Table 1). During the course of PD, two patients developed dementia (one Italian patient and the Dutch patient II-2 - Family 2), two had no cognitive impairments (French patients), and one had multiple depressive periods (Dutch patient II-2 - Family 1).

Limited data are available for the p.Arg263His and p.Arg151Cys variants reported here in two patients with PD and PDD, respectively. Besides being very rare, predicted to be pathogenic by the majority of *in-silico* programs used here, and to replace highly conserved

amino acids located within conserved protein stretches (Supplementary Figure 2), no additional affected carriers have been reported so far, and functional data are not available. Therefore pathogenicity cannot be confidently established. Of note, the p.Arg151Cys substitution was previously found by us[9] in one of 645 Dutch patients with abdominal aortic aneurysms. However, the neurological status of this subject is unknown.

In conclusion, we report *LRP10* possibly pathogenic variants in patients with PD, PDD and dementia from a local Dutch population. Although our data cannot conclusively prove pathogenicity, the identification of additional patients with PD, PDD and dementia carrying the *LRP10* p.Tyr307Asn variant provides further evidence that this variant might be pathogenic.

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Supplementary Information

Sanger sequencing of LRP10

Amplification of DNA fragments was performed in a total volume of 20 µl, containing 2.0 µl of 10X FastStart Taq DNA Polymerase buffer, 1.6 µl of 2.5 mM dNTPs, 1.0 µl of 10 µM forward primer, 1.0 µl of 10 µM reverse primer, 0.10 or 0.15 µl of FastStart Taq. DNA Polymerase (Roche, Basel, Switzerland), and 25 ng of genomic DNA. In addition, 4 µl of 1X GC-RICH solution (Roche) was used for exon 1, 5 and 7. We used the same primers that have been previously reported by us.[1] Initial denaturation was performed for 5 minutes at 94˚C, followed by 30 cycles of 30 seconds at 94˚C, 30 seconds at 60˚C, and 90 seconds at 72˚C. The final extension step was done for 5 minutes at 72˚C. Removal of unconsumed dNTPs and primers was performed with 3µl of PCR product, using 5 units of ExoI and 0·5 unit of Fast AP (Thermo Fisher Scientific, Waltham, MA, USA) for 45 minutes at 37˚C and 15 minutes at 80˚C. DNA strands were sequenced directly using the Big Dye Terminator (version 3·1; Thermo Fisher Scientific) according to the manufacturer's protocol. SephadexG50 (GE Healthcare, Little Chalfont, UK) was used to remove dye terminators. Fragments were loaded on an ABI 3730XL Genetic Analyzer (Thermo Fisher Scientific). Sequences were analyzed using the software packages Seqscape v3·0 (Thermo Fisher Scientific) and Sequencing Analysis v6·0 (Thermo Fisher Scientific). For annotating *LRP10* sequence variants transcript NM_0140445.4 was adopted and the Human Genome Variation Society (HGVS) nomenclature was applied to describe the sequence variants.[2]

Whole exome sequencing and copy number analysis of LRP10 variant carriers

Whole exome sequencing (WES) was performed in the possibly pathogenic *LRP10* variant carriers by Nimblegen SeqCap EZ MedExome 47 Mb in combination with Illumina Paired-End Library Preperation and 2x 150 bp Sequencing on an Illumina HiSeq2000 sequencer. Using standard procedures[3], reads were aligned to the human reference genome (hg19) using the Burrows-Wheeler alignment[4] tool and processed using Picard (http://broadinstitute.github.io/picard) and the Genome Analysis Toolkit (GATK)[5]. GATKs Haplotype Caller was used for variant calling and ANNOVAR for the annotation (version 2018Apr16)[6].

To screen for copy number variants of *SNCA*, *PARK7*, *PINK1*, and *PARK2*, Multiplex Ligation-dependent Probe Amplification (MLPA) was performed. The P051-D1 Parkinson kit (MRC Holland) was used according to the manufacturer's protocol. Analysis was done using an ABI 3730XL Genetic Analyzer (Thermo Fisher Scientific) and Seqscape v3·0 (Thermo Fisher Scientific).

Haplotype analysis

Nine short tandem repeat (STR) markers located within a ~6 Mb genomic region containing the *LRP10* locus were genotyped. PCR reactions were performed in a total volume of 20 µl, containing 30 ng of genomic DNA, 1x PCR buffer (FastStart, Roche), 200 µM dNTPs, 0.5 µM fluorescent forward primer, 0.5 µM reverse primer, and 0.5 unit FastStart Taq Polymerase (Roche). Primers for the amplification of markers D14S261, D14S283 and D14S275 were taken from the available ABI Prism Linkage Mapping Set Version 2.5. Details of the other primers used for STR markers genotyping are available in Supplementary Table 5. PCR was carried out following an initial denaturation at 94˚C for 4 minutes, followed by 30 cycles of 30 seconds at 94˚C, 30 seconds at 60˚C, and 90 seconds at 72˚C. The final extension step was done for 7 minutes at 72˚C. Fluorescent PCR products were mixed with GeneScan 500-LIZ Size Standard (Applied Biosystems), loaded on an ABI 3730XL automated sequencer (Thermo Fisher Scientific), and analyzed using GeneMarker v.2.4.0 software package (SoftGenetics, State College, PA, USA).

Supplementary Table 1: Genes with established or proposed association with PD or parkinsonism.

Chr	Start	End	Gene	Mode of inheritance
1	8021714	8045342	*PARK7*	AR
1	17312453	17338467	*ATP13A2*	AR
1	20959948	20978004	*PINK1*	AR
1	65720133	65881552	*DNAJC6*	AR
2	25013136	25016251	*PTRHD1*	AR
6	161768590	163148834	*PARK2*	AR
15	62144588	62352664	*VPS13C*	AR
21	33997269	34100351	*SYNJ1*	AR
22	32870707	32894818	*FBXO7*	AR
22	38507502	38577857	*PLA2G6*	AR
1	11072462	11085549	*TARDBP*	AD
1	155204239	155214653	*GBA**	AD
3	132136361	132257876	*DNAJC13*	AD
4	90645250	90759447	*SNCA*	AD
7	56169266	56174187	*CHCHD2*	AD
12	40618813	40763087	*LRRK2*	AD
14	23340822	23350789	*LRP10*	AD
16	46693589	46723144	*VPS35*	AD
17	42422491	42430474	*GRN*	AD
17	43971702	44105700	*MAPT*	AD
20	5049129	5093736	*TMEM230*	AD
X	154487526	154493852	*RAB39B*	X-linked R

* Heterozygous variants in *GBA* are known risk factors for PD. GRCh37: genome reference consortium human 37. AR: autosomal recessive, AD: autosomal dominant, PD: Parkinson's disease.

Supplementary Table 2: Demographic and clinical characteristics of the patients included in the study.

Sex, male		81 (62%)
Diagnosis at baseline	PD	130 (100%)
Age at baseline, years		69.9 (8.4)
Age at onset, years		62.4 (9.6)
MMSE at baseline*		26.8 (3.8)
Family history of PD	only 1st-degree	21 (16%)
	only 2nd-degree	13 (10%)
	1st- and 2nd-degree	3 (2%)
	absent	93 (72%)
Family history of dementia	only 1st-degree	31 (23%)
	only 2nd-degree	8 (6%)
	1st and 2nd-degree	4 (3%)
	absent	87 (68%)
Family history of PD and/or dementia	1st- and/or 2nd-degree	68 (52%)
Diagnosis at follow up	PD	71 (55%)
	PDD	55 (42%)
	DLB	4 (3%)
Follow up, years		7.0 (3.2)
N. of deceased patients		70 (54%)
Disease duration**, years		14.0 (8.3)

Data are presented as mean (SD) or n (%).*MMSE data was available in 128 patients; ** Data on disease duration was available in 70 patients. MMSE: Mini Mental State Examination, PD: Parkinson's disease, PDD: Parkinson's disease dementia, DLB: dementia with Lewy bodies.

Supplementary Table 3: Additional *LRP10* variants identified in the study.

N. of patients	Genomic position	Nucleotide change	Amino acid change	Exon / Intron	Coding effect	dbSNP accession number	MAF GnomAD (N. of alleles)	Functional predictions: pathogenic (total)	Splicing predictions: deleterious (total)
1	14:23341969	c.57C>T	p.Asp19=	exon 2	synonymous	rs772482762	0.004% (11)	NA	0/5
1	14:23346279	c.1685G>A	p.Arg562His	exon 7	missense	rs142153001	0.70% (1974)	9/10	n.a.
2	14:23342497	c.80-23G>A	n.a.	intron 2	n.a.	rs145476957	0.69% (1950)	NA	n.a.
1	14:23345195	c.1038 C>T	p.Asp346=	exon 5	synonymous	rs201657631	0.007% (20)	NA	0/2

GRCh37: genome reference consortium human 37; NM_014045.4. MAF: minor allele frequency, GnomAD: Genome Aggregation Database, NA: not available, n.a.: not applicable. Splicing prediction programs for p.Asp19=: SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer, and Human Splicing Finder integrated in Alamut Visual version 4.2 (Interactive Biosoftware, Rouen, France). Splicing prediction programs for p.Asp346= : Berkeley Drosophila Genome Project Searches Splice Site prediction by Neural Network http://www.fruitfly.org/seq_tools/splice.html; NatGene2 Server http://www.cbs.dtu.dk/services/NetGene2/.

Supplementary Table 4: *In-silico* pathogenicity predictions for the *LRP10* variants.

				***In-silico* predictions** (scores)										
Genomic position	**Nucleotide change**	**Amino acid change**	**GERP**	**SIFT**	**Polyphen2 HDIV**	**Polyphen2 HVAR**	**LRT**	**Mutation Taster**	**Mutation Assessor**	**FATHMM**	**Meta-SVM**	**Meta-LR**	**CADD phred**	**M-CAP**
14:23345076	c.919T>A	p.Tyr307Asn	5.97	D (0.0)	D (0.999)	D (0.942)	D (0.000)	D (1.000)	M (2.525)	D (-3.32)	D (0.771)	D (0.848)	26.0	D (0.140)
14: 23344945	c.788G>A	p.Arg263His	5.97	D (0.02)	D (1.0)	D (0.927)	D (0.000)	D (0.975)	L (1.7)	T (2.25)	T (-1.146)	T (0.096)	29.1	T (0.008)
14:23344608	c.451C>T	p.Arg151Cys	5.01	D (0.0)	P (0.472)	B (0.037)	D (0.000)	D (1.000)	M (2.285)	D (-3.92)	D (0.545)	D (0.777)	32	D (0.172)
14:23341969	c.57C>T	p.Asp19=	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14:23346279	c.1685G>A	p.Arg562His	5.23	T (0.68)	D (0.999)	D (0.972)	D (0.000)	D (1.000)	M (2.2)	D (-3.37)	D (0.451)	D (0.772)	26.6	NA
14:23342497	C.80-23G>A	n.a.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14:23345195	c.1038 C>T	p.Asp346=	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

GERP: Genomic Evolutionary Rate Profiling, SIFT: Sorting Intolerant From Tolerant, PolyPhen2 HDIV: Polymorphism Phenotyping version 2 human diversity, PolyPhen2 HVAR: Polymorphism Phenotyping version 2 human variation, LRT: Likelihood Ratio Test, FATHMM: Functional Analysis Through Hidden Markov Models, SVM: Support Vector Machine, LR: Logistic Regression, CADD: Combined Annotation Dependent Depletion, M-CAP: Mendelian Clinically Applicable Pathogenicity, T: tolerated, D: damaging/ disease causing, B: benign, N: polymorphism/neutral, P: polymorphism automatic, L: low, M: medium, PP: probably pathogenic, LB: likely benign, NA: not available, n.a.: not applicable. Transcript references: NM_014045.4. Annovar (version 2018Apr16).

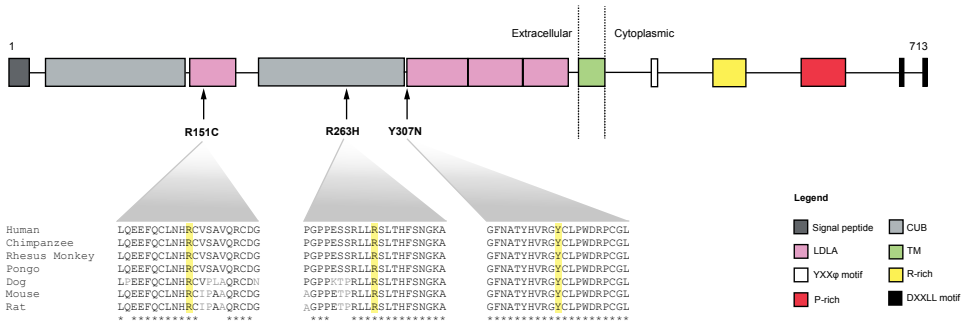
Supplementary Table 5: PCR Primers for markers genotyping.

Marker	Oligo Name	Oligo Sequence
CGR6800	CGR6800-FAM-FWD	5'-TGCCTGGCAAAACACACACAC-3'
	CGR6800-REV	5'-GGCTGAGGCAGGACAATCAC-3'
CGR4118	CGR4118-FAM-FWD	5'-ATTTCCAGCCTCCCTCTAGCC-3'
	CGR4118-REV	5'-GCCCAGTGTCTGGGGAGTAGG-3'
CGR7011	CGR7011-FAM-FWD	5'-GGCCACATTCGACTGTCATAGC-3'
	CGR7011-REV	5'-CTTCCAAGCCGACAGGATGG-3'
D14S1023	D14S1023-FAM-FWD	5'-AAAGGACCTCACAAATTCCTTCTAGC-3'
	D14S1023-REV	5'-TCTTGATAGTCTTAAGGTAGCAACAACAGC-3'
D14S990	D14S990-FAM-FWD	5'-ATATTGGGGGTGGGCTGTGG-3'
	D14S990-REV	5'-GCTGAATAAAGTTGCACTGTGACTGG-3'
D14S972	D14S972-FAM-FWD	5'-GAGGTACAAGAAACTTAGAGAACCTCAAGC-3'
	D14S972-REV	5'-TGTCTACAGATTCAATGCAATACTAACAGG-3'

Primers for the amplification of markers D14S261, D14S283 and D14S275 were according to the ABI Prism Linkage Mapping Set Version 2.5.

	Family 1						Family 2						Italian Patient*		Genomic Position (bp)
	II-2		III-1		III-2		II-2		II-3		III-2				
D14S261	299	297	297	297	297	297	301	297	301	297	297	297	295	303	20,472,545
D14S1023	296	294	296	294	296	294	292	294	292	294	294	294	296	294	21,074,061
D14S283	135	129	129	129	129	129	149	129	149	129	149	141	149	149	22,319,885
CGR6800	294	294	294	294	298	294	294	294	294	294	294	292	294	294	22,826,800
LRP10 p.Tyr307Asn	T	A	T	A	T	A	T	A	T	A	T	T	T	A	22,881,580
CGR4118	390	390	390	390	390	390	390	390	390	390	390	390	392	390	22,884,118
CGR7011	270	270	270	270	290	270	290	270	290	270	290	270	290	270	22,997,011
D14S990	201	207	201	207	195	207	191	207	191	207	191	199	193	199	23,217,423
D14S972	257	255	253	255	253	255	253	251	253	251	253	253	251	253	23,978,736
D14S275	153	149	155	149	153	149	155	151	155	151	155	153	149	155	26,327,814

Supplementary Figure 1: Haplotype analysis.
Haplotype analysis of the *LRP10* region in two Dutch families (genealogically related within six generations) and one Italian patient reported previously with the same *LRP10* c.919T>A, p.Tyr307Asn variant (* Patient II-1, in Quadri et al., 2018); for pedigree position refer to Figure 1 in the manuscript. All *LRP10* p.Tyr307Asn variant carriers share three markers flanking the variant, displayed in red. The individuals from the two Dutch families share a longer haplotype (displayed in orange), in keeping with a more recent common founder. Other markers specific to each family are reported in blue and green. Genomic positions are according to the Genome Reference Consortium human genome build 38 (GRCh38).



Supplementary Figure 2: *LRP10* protein structure and variants conservation analysis.
Representation of LRP10 protein structure and conservation analysis between LRP10 orthologs of p.Arg151, p.Arg263 and p.Tyr307 amino acids. CUB: compement C1R/C1S, urchin EGF, BMP1, LDLA: low-density lipoprotein receptor class A, TM: transmembrane domain, R-rich: Arginine-rich domain, YXXφ: a motife of Tyrosine plus two other amino acids, then an amino acid with a large bulky hydrophobic side chain, P-rich: Roline-rich domain, DXXLL: a motif of an aspartic acid, two other amino acids, then two leucines. We used the following National Centre for Biotechnology Information (NCBI) LRP10 Reference Sequence: Human, Homo Sapiens NP_054764.2; Chimpanzee, Pan troglodytes XP_509843; Rhesus monkey, Macaca mulatta NP_001244860.1; Pongo, Sumatran orangutan NP_001125058; Dog, Canis lupus familiaris XP_537364; Mouse, Mus musculus NP_075369; Rattus norvegicus NP_001032866.

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4.3

Chapter 4.3

LRP10 variants in progressive supranuclear palsy

Leonie J.M. Vergouw, Shamiram Melhem, Laura Donker Kaat, Wang Z. Chiu, Demy J.S. Kuipers, Guido Breedveld, Agnita J.W. Boon, Li-San Wang, Adam C. Naj, Elizabeth Mlynarksi, Laura Cantwell, Marialuisa Quadri, Owen A. Ross, Dennis W. Dickson, Gerard D. Schellenberg, John C. van Swieten, Vincenzo Bonifati and Frank Jan de Jong

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Abstract

The aim of this study was to explore whether variants in *LRP10*, recently associated with Parkinson's disease and dementia with Lewy bodies, are observed in two large cohorts (discovery and validation cohort) of patients with progressive supranuclear palsy (PSP). A total of 950 PSP patients were enrolled: 246 PSP patients (n=85 possible (35%), n=128 probable (52%), n=33 definite (13%)) in the discovery cohort, and 704 patients with definite PSP in the validation cohort. Sanger sequencing of all *LRP10* exons and exon-intron boundaries was performed in the discovery cohort, and whole exome sequencing was performed in the validation cohort. Two patients from the discovery cohort and eight patients from the validation cohort carried a rare, heterozygous, possibly pathogenic *LRP10* variant (p.Gly326Asp, p.Asp389Asn, and p.Arg158His, p.Cys220Tyr, p.Thr278Ala, p.Gly306Asp, p.Glu486Asp, p.Arg554*, p.Arg661Cys). In conclusion, possibly pathogenic *LRP10* variants occur in a small fraction of PSP patients and may be overrepresented in these patients compared to controls. This suggests that possibly pathogenic *LRP10* variants may play a role in the development of PSP.

Introduction

Progressive supranuclear palsy (PSP) is an adult-onset, progressive neurodegenerative disorder clinically characterized by parkinsonism, vertical supranuclear gaze palsy, and postural instability with falls. Other PSP features include frontal lobe and bulbar dysfunction, and cognitive decline.[1,2] The clinical presentation of PSP is heterogeneous, and ten different clinical phenotypes have been described in patients with PSP neuropathology.[3] PSP brain pathology includes neurofibrillary tangles, neutrophil threads, tufted astrocytes, neuronal loss and gliosis in multiple subcortical areas and other regions.[4]

PSP is usually considered a sporadic tauopathy of unknown etiology.[5] However, rare familial forms have been reported.[6,7] Mutations in the *microtubule-associated protein tau* (*MAPT*) gene have been reported as the likely disease cause in a few pathologically confirmed PSP patients.[8,9] Furthermore, genome-wide association studies have shown associations between PSP and the *MAPT*, *syntaxin-6* (*STX6*), *myelin-associated oligodendrocyte basic protein* (*MOBP*), and *eukaryotic translation initiation factor 2-alpha kinase* (*EIF2AK*) genes, modulating the risk of developing PSP.[10,11] Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) gene have also been implicated in a small number of PSP cases[12,13] and are the most common genetic cause of Parkinson's disease (PD)[14]. Interestingly, pleomorphic neuropathology has been observed in PD patients with *LRRK2* mutations, ranging from typical alpha-synuclein-positive pathology (seen in most cases), to PSP-like pathology.[15] Indeed in the original Japanese family, which nominated the linkage region for *LRRK2*, the affected members presented with tauopathy.[16] Because of these genetic and pathological overlaps between PD and PSP, we hypothesized that variants in the *low-density lipoprotein receptor related protein 10* (*LRP10*) gene, recently associated with PD and dementia with Lewy bodies (DLB)[17,18], might also be implicated in PSP. *LRP10* is a surface protein, which function is largely elusive. However, some studies have suggested a role of LRP10 in ligand trafficking between the trans-Golgi network, endosomes, and the plasma membrane. Furthermore, LRP10 has been linked to the metabolism of amyloid-β and α-synuclein.[19,20,21]

The aim of this study is to explore whether possibly pathogenic variants in *LRP10* are observed in a large Dutch cohort of PSP patients (discovery cohort) using Sanger sequencing. Additionally, we try to validate our findings in a large cohort of PSP patients (validation cohort) from the USA and Europe using whole exome sequencing (WES).

Methods

Subjects

PSP patients from two large cohorts were enrolled in this study. PSP was diagnosed according to the criteria of the National Institute for Neurological Disorders and Stroke / Society for PSP (NINDS-SPSP)[1]. The discovery cohort consisted of 246 PSP patients enrolled from a large Dutch cohort[22], consecutively collected between 2003 and 2012 (Table 1). Patients were ascertained at the outpatient clinic of the Erasmus Medical Center Rotterdam, at home or at nursing homes. At inclusion, information about patient's medical and family history and current medical status was collected. Furthermore, neurological examination was performed and a blood sample was collected. The validation cohort consisted of 704 neuropathologically confirmed PSP patients enrolled form a large cohort of patients from the USA and Europe[23] (Table 1). These patients were identified from brain banks, research hospitals and neuropathologists. The study was approved by the relevant Institutional Ethical Authorities and all participants or legal representatives signed informed consent.

Table 1: Demographic and clinical characteristics.

		n=246	**n=704**
Sex, male		127 (52%)	377 (54%)
Diagnosis (NINDS-SPSP)	possible PSP	85 (35%)	0 (0%)
	probable PSP	128 (52%)	0 (0%)
	definite PSP	33 (13%)	704 (100%)
Age at onset, years (n=246;476)		65.8 (7.5)	68.1 (8.4)
Family history of neurodegenerative diseases (n=244;0)	1st-degree	71 (29%)	NA
	2nd-degree	21 (9%)	NA
	no	152 (62%)	NA
Deceased (n=244;704)		242 (98%)	704 (100%)
Age at death, years (n=241;698)		73.7 (7.3)	75.3 (8.2)

Data are presented as n (%) or mean (SD). NINDS-SPSP: National Institute for Neurological Disorders and Stroke / Society for PSP, PSP: progressive supranuclear palsy, NA: not available.

Genetic analyses

Genetic analyses in the two cohorts

Genomic DNA was isolated from blood in the discovery cohort and from brain tissue in the validation cohort using standard methods. Sanger sequencing was performed for the entire open reading frame and exon-intron boundaries of *LRP10* in the discovery cohort (protocol reported by Vergouw et al., 2019[18]). WES was performed in the validation cohort (Supplementary Information). Possibly pathogenic *LRP10* variants identified by WES in the validation cohort were validated by Sanger sequencing (Supplementary Information).

We considered variants as possibly pathogenic according to the following criteria: (1) heterozygous state; (2) rarity, defined as a frequency <0.1% in the Genome Aggregation Database (GnomAD v2.1); (3) exonic location and non-synonymous, or predicted to affect splicing; and (4) predicted as pathogenic by at least five of 11 *in-silico* programs (Supplementary Information).

Additional genetic analyses in possibly pathogenic LRP10 variant carriers in the discovery cohort

WES, multiple ligation-dependent probe amplification (MLPA, P051-Parkinson mix 1), and *C9orf72* repeat expansion analysis were performed in patients who carried possibly pathogenic *LRP10* variants to exclude possibly pathogenic variants in other known genes causing parkinsonism or dementia (Supplementary Table 1). The presence of possibly pathogenic variants in known genes causing parkinsonism or dementia in possibly pathogenic *LRP10* variant carriers decreases the chance of the *LRP10* variant to be truly pathogenic. WES and MLPA were performed as reported previously by Vergouw et al., 2019[18]. Details of the methods of the *C9orf72* repeat expansion analysis can be found in the Supplementary Information.

Results

Demographic and clinical characteristics of the two cohorts

The discovery cohort consisted of 85 (35%) patients with possible PSP, 128 (52%) with probable PSP (52%), and 33 (13%) with definite PSP. The mean disease onset age in this cohort was 65.8 ± 7.5 years and 52% of patients were male; 29% of patients had at least one first-degree relative and 9% had at least one second-degree relative with a neurodegenerative disease. The validation cohort consisted of 704 definite PSP patients. The mean disease onset age in this cohort was 68.1 ± 8.4 years (data only available in n=476) and 54% of patients were male (Table 1).

Genetic findings

Two possibly pathogenic *LRP10* variants were detected in the discovery cohort, each in single patients (p.Gly326Asp and p.Asp389Asn). In the validation cohort, seven possibly pathogenic *LRP10* variants were detected in eight patients (p.Arg158His, p.Cys220Tyr, p.Thr278Ala, p.Gly306Asp, p.Glu486Asp, p.Arg554*, and p.Arg661Cys; see Table 2 and Supplementary Table 2 for specifications). Supplementary Figure 1a shows the *LRP10* gene structure with the location of the identified variants and Supplementary Figure 1b shows the LRP10 protein structure with the location of the amino acid changes. Other variants in *LRP10* which did not fulfill the criteria for possible pathogenicity, as described in section 2.2.1., are depicted in Supplementary Table 3. Additional WES analysis (average depth of >170x with 99% of the target region covered >20x) in the possibly pathogenic *LRP10* variant carriers

Table 2: Possibly pathogenic *LRP10* variants.

	Genetic information									Clinical information			
	Genomic position	**Nucleotide change**	**Amino acid change**	**Exon**	**Coding effect**	**dbSNP 142 accession number**	**Allele frequency GnomAD (alleles)**	**Functional predictions: pathogenic (total)**	**Splicing predictions: deleterious (total)**	**Patient**	**NINDS-SPSP criteria**	**Age at onset (years)**	**Age at death (years)**
Discovery cohort													
	14:23345322	c.1165G>A	p.Asp389Asn	5	missense	rs754181235	0.01% (37)	6/11	n.a.	1	Probable PSP	61	66
	14:23345134	c.977G>A	p.Gly326Asp	5	missense	rs547591765	0.006% (14)	6/11	n.a.	2	Possible PSP	55	65
Validation cohort													
	14:23344630	c.473G>A	p.Arg158His	5	missense	rs764424911	0.005% (13)	6/11	n.a.	1	Definite PSP	68	75
	14:23344816	c.659G>A	p.Cys220Tyr	5	missense	rs867533372	.	10/11	n.a.	2	Definite PSP	70	74
	14:23344989	c.832A>G	p.Thr278Ala	5	missense	.	.	5/11	n.a.	3	Definite PSP	74	87
	14:23345074	c.917G>A	p.Gly306Asp	5	missense	rs375748692	0.007% (21)	9/11	n.a.	4	Definite PSP	NA	66
										5	Definite PSP	74	80
	14:23345931[1]	c.1458G>C	p.Glu486Asp	6	missense	rs142130715	0.01% (32)	11/11	0/4	6	Definite PSP	78	83
	14:23346254	c.1660C>T	p.Arg554*	7	stopgain	rs201213246	0.01% (28)	n.a.	n.a.	7	Definite PSP	74	84
	14:23346575	c.1981C>T	p.Arg661Cys	7	missense	rs771796662	0.004% (10)	8/11	n.a.	8	Definite PSP	NA	84

The Genome Reference Consortium Human Build 37 (hg19) and NM_014045-4 LRP10 transcript were used. * This variant was not validated by Sanger sequencing, because no additional DNA was available. GnomAD: Genome Aggregation Database, NINDS-SPSP: National Institute for Neurological Disorders and Stroke / Society for progressive supranuclear palsy, n.a.: not applicable, NA: not available. Splicing prediction programs: SSF, MaxEnt, NNSPLICE, Gene Splicer.

from the discovery cohort revealed a heterozygous *VPS13C* variant (p.Gln2546*, absent in GnomAD v2.1) in one patient (Supplementary Table 4). No other mutations in genes causing parkinsonism or dementia were found.

Clinical information of possibly pathogenic LRP10 variant carriers

An overview of the clinical information of the possibly pathogenic *LRP10* variant carriers is shown in Table 2. Patient 1 from the discovery cohort (*LRP10* p.Asp389Asn variant) experienced falls from the age of 61, followed by swallowing problems. At the age of 63, a mild downward vertical supranuclear gaze palsy, dysarthric speech, reduced arm swing, palatal tremor and impaired balance, but no clear ataxia were observed. He had a favorable response to levodopa. At neuropsychological examination deficits were observed in attention, concentration and executive functioning. Furthermore, mild memory and naming problems were seen. Brain MRI showed mild parieto-occipital and cerebellar atrophy and hypertrophy of the olivary nuclei. The patient died at the age of 66. Family history was negative for parkinsonism, dementia or motor neuron disease. Brain autopsy was not performed. This patient was diagnosed with probable PSP during life according to the NINDS-SPSP criteria[1] and can retrospectively be classified as probable PSP with Richardson's syndrome (PSP-RS) according to the MDS criteria[23]. Patient 2 from the discovery cohort (*LRP10* p.Gly326Asp variant) experienced tremor of the right leg from the age of 55, followed by falls, rigidity, swallowing, speech and memory problems from the age of 60. At the age of 64, vertical supranuclear gaze palsy, bradykinesia, intermittent rest tremor of arms and legs and balance problems were observed. She had a favorable response to levodopa. Neuropsychological examination showed severe deficits, especially with frontal subcortical and language problems. Brain MRI was unremarkable. The patient died at the age of 65. Family history was negative for parkinsonism, dementia or motor neuron disease. Brain autopsy was not performed. This patients was diagnosed with possible PSP during life according to the NINDS-SPSP criteria[1] and can retrospectively be classified as probable PSP with predominant parkinsonism (PSP-P) according to the MDS criteria[23].

Discussion

In this study we explored the presence of *LRP10* variants in two cohorts with a total of 950 PSP patients (discovery cohort n=246, validation cohort n=704). The PSP diagnosis was pathologically confirmed in 78% of these patients. Two possibly pathogenic *LRP10* variants (p.Gly326Asp and p.Asp389Asn) were identified in two patients from the discovery cohort and seven possibly pathogenic *LRP10* variants (p.Arg158His, p.Cys220Tyr, p.Thr278Ala, p.Gly306Asp, p.Glu486Asp, p.Arg554*, and p.Arg661Cys) were identified in eight patients from the validation cohort. These variants are very rare, are predicted to be pathogenic by ≥5 *in-silico* programs and are mostly located in *LRP10* exon 5, where other probably pathogenic variants were previously found[17]. Interestingly, the frequency of possibly pathogenic *LRP10*

variants is significantly higher in the validation cohort (8/1408 alleles=0.6%) compared to a previous published control cohort of patients with abdominal aneurysms[17] (1/1248 alleles=0.08%; Fishers's Exact test *p* value 0.04). In addition, the p.Gly306Asp variant has been identified previously in two out of 2835 PD patients and one out of 5343 controls[24], the p.Gly326Asp variant in one out of 264 patients with multiple system atrophy and in no controls[25], and the p.Glu486Asp variant in three out of 2835 PD patients and one out of 111 DLB patients compared to none in 5343 and 233 controls, respectively[24]. The p.Arg158His, p.Arg554* and p.Arg661Cys variants have previously been identified in single controls[24-26] (Supplementary Table 5).

Both patients from the discovery cohort displayed uncommon PSP clinical features. Patient 1 had a palatal tremor, inferior olivary hypertrophy and cerebellar atrophy. Inferior olivary hypertrophy is observed in 1.5% of pathologically confirmed PSP patients[27], but associated palatal tremor is very rare in PSP[27,28]. The syndrome of progressive ataxia and palatal tremor[29] may retrospectively also be considered in patient 1, yet the clinical phenotype is most consistent with PSP. An uncommon feature in patient 2 was the presence of an isolated tremor of the right leg in the first 5 years of the disease. Unfortunately, autopsy studies were not performed in these patients, and therefore the diagnosis could not be verified at pathological level. The absence of a family history of PSP or other neurodegenerative disorders in these two patients would be compatible with an incomplete penetrance or a de novo occurrence of the *LRP10* variants.

Of note, a *VPS13C* variant (p.Gln2546*) was observed in one *LRP10* variant carrier. Mutations in *VPS13C* are associated with autosomal recessive forms of early-onset parkinsonism.[30] In our patient the variant was found in heterozygous state and is therefore most likely an incidental finding.

Strengths of this study are the large sample size of the two PSP cohorts, the validation of our findings in an independent cohort, and the high percentage of neuropathologically confirmed PSP patients. Limitations are the lack of screening for *LRP10* genomic deletions of multiplications (not detectable by Sanger methods).

In conclusion, this is the first study of *LRP10* in two large PSP cohorts. We showed that rare, possibly pathogenic *LRP10* variants occur in a small, but substantial fraction of PSP patients. Furthermore, possibly pathogenic *LRP10* variants may be overrepresented in PSP patients compared to controls and may therefore play a role in disease pathogenesis. Further studies are warranted to replicate our findings and to study which molecular mechanisms underlie the possible association between *LRP10* and PSP.

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Supplementary Information

Whole exome sequencing in validation cohort

Whole exome sequencing (WES) was performed using the Nimblegen's VCRome v2.1 (36Mb) capture kit on an Illumina HiSeq sequencer. Sequencing data were analyzed using the in-house DNA Resequencing analysis workflow (DRAW)[1]. Reads were aligned to the human reference genome (hg19) using the Burrows-Wheeler alignment tool (0.7.5)[2] and processed using Picard (http://picard.sourceforge.net/) and the Genome Analysis Toolkit (GATK)[3]. Additional sequencing was done for samples where coverage did not reach 20x for more than 80% of the targeted region and 10x for more than 90% of the targeted regions. Data from multiple sequencing experiments from the same individual were merged using SAMtools[4]. Variant calling was performed by GATKs Haplotype Caller and annotated using SNPEff[5]. An average depth of 33x was reached.

Sanger sequencing in validation cohort

Genomic DNA (~50ng) was amplified using a SimpliAmp Thermal Cycler (Applied Biosystems) in a 20 µl reaction volume with HotStarTaq Master Mix (Qiagen) in the presence of 2 µM primers (IDT). The PCR conditions used were: 95°C 15min followed by 30 cycles of 95°C 20sec, 55°C 30sec, 72°C 2min with a final extension of 72°C 7min. The amplified PCR products were prepared for Sanger sequencing by adding ExoSAP-IT (USB) and incubating at 37°C for 45min followed by 80°C for 15min. The PCR products were then Sanger sequenced using the BigDye® Terminator v3.1 Cycle Sequencing kit (Part No. 4336917 Applied Biosystems). The sequencing reaction contained BigDye® Terminator v3.1 Ready Reaction Mix, 5X Sequencing Buffer, 5M Betaine solution (Part No. B0300 Sigma) and 0.64uM sequencing primer (IDT) in a total volume of 5ul. The sequencing reaction was performed in a SimpliAmp Thermal Cycler (Applied Biosystems) using the following program: 96°C 1min followed by 25 cycles of 96°C 10sec, 50°C 5sec, 60°C 1min15sec. The products were cleaned using XTerminator and SAM Solution (Applied Biosystems) with 30 min of shaking at 1800 rpm followed by centrifugation at 1000 rpm for 2min. The sequencing products were analyzed on a 3130XL Genetic Analyzer (Applied Biosystems) and the sequencing traces were analyzed using Sequencer 5.4 (Gene Code). Information on primers can be given by the Authors on request.

C9orf72 repeat expansion analysis

C9orf72 repeat expansion analysis was performed to screen for the presence of a pathogenic chromosome 9p21 GGGGCC hexanucleotide repeat expansion. A previously described repeat-primed PCR assay[6] was used. An ABI3730XL Genetic Analyzer (Thermo Fisher Scientific) and GeneMarker Ver.2.4.0 (SoftGenetics, State College, PA, USA) were used for analysis. A pathogenic *C9orf72* expansion was defined as more than 30 repeats.[6]

Supplementary Table 1: Known genes causing parkinsonism or dementia.

Chr	Start	End	Gene	Mode of inheritance
			PD genes	
1	8021714	8045342	*PARK7*	AR
1	17312453	17338467	*ATP13A2*	AR
1	20959948	20978004	*PINK1*	AR
1	65720133	65881552	*DNAJC6*	AR
2	25013136	25016251	*PTRHD1*	AR
6	161768590	163148834	*PARK2*	AR
15	62144588	62352664	*VPS13C*	AR
21	33997269	34100351	*SYNJ1*	AR
22	32870707	32894818	*FBXO7*	AR
22	38507502	38577857	*PLA2G6*	AR
1	11072462	11085549	*TARDBP*	AD
1	155204239	155214653	*GBA**	AD
3	132136361	132257876	*DNAJC13*	AD
4	90645250	90759447	*SNCA*	AD
7	56169266	56174187	*CHCHD2*	AD
12	40618813	40763087	*LRRK2*	AD
14	23340822	23350789	*LRP10*	AD
16	46693589	46723144	*VPS35*	AD
17	42422491	42430474	*GRN*	AD
17	43971702	44105700	*MAPT*	AD
20	5049129	5093736	*TMEM230*	AD
X	154487526	154493852	*RAB39B*	X-linked R
			FTD genes	
1	11072462	11085549	*TARDBP*	AD
3	87276413	87304698	*CHMP2B*	AD
7	144149034	144533488	*TBK1*	AD
9	35056065	35072739	*VCP*	AD
16	31191431	31206192	*FUS*	AD
17	43971702	44105700	*MAPT*	AD
17	42422491	42430474	*GRN*	AD
X	56590025	56593443	*UBQLN2*	X-linked D
1	227057885	227083804	**AD genes**	AD
11	121322912	121504471	*PSEN2*	AD
14	73603143	73690399	*SORL1*	AD
19	1040102	1065571	*PSEN1*	AD
21	27252861	27543446	*ABCA7**	AD
			APP	
			Perry syndrome gene	
2	74588281	74619214	*DCTN1*	AD
			Niemann-Pick C genes	AR
14	74942900	74960084	*NPC2*	AR
18	21086148	21166581	*NPC1*	

* also risk gene. The Genome Reference Consortium Human Build 37 (hg19) was used. AR: autosomal recessive, AD: autosomal dominant or Alzheimer's disease, PD: Parkinson's disease, FTD: frontotemporal dementia.

Supplementary Table 2: *In-silico* pathogenicity predictions.

					Variant-effect predictions software (scores)										
	Genomic position	**Nucleotide change**	**Amino acid change**	**GERP**	**SIFT**	**Polyphen2 HDIV**	**Polyphen2 HVAR**	**LRT**	**Mutation Taster**	**Mutation Assessor**	**FATHMM**	**MetaSVM**	**MetaLR**	**CADD phred**	**M-CAP**
Discovery cohort	14:23345134	c.977G>A	p.Gly326Asp	4.14	T (0.368)	B (0.297)	B (0.172)	N (0.009)	D (0.887)	L (1.245)	D (-3.23)	D (0.067)	D (0.644)	15.59	D (0.034)
	14:23345322	c.1165G>A	p.Asp389Asn	4.9	T (0.584)	B (0.022)	B (0.007)	D (0.000)	D (1.000)	L (1.165)	D (-2.67)	T (-0.226)	D (0.535)	17.57	D (0.030)
	14: 23341951	c.39C>T	p.Gly13=	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14:23345195	c.1038 C>T	p.Asp346=	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14:23346279	c.1685G>A	p.Arg562His	5.23	T (0.68)	D (0.999)	D (0.972)	D (0.000)	D (1.000)	M (2.2)	D (-3.37)	D (0.451)	D (0.772)	26.6	NA
	14:23346630	c.2036A>C	p.His679Pro	3.84	T (0.143)	B (0.072)	B (0.037)	N (0.226)	D (1.000)	L (0.975)	D (-3.05)	T (-0.202)	D (0.561)	9.054	D (0.093)
Validation cohort	14:23344630	c.473G>A	p.Arg158His	5.03	T (0.064)	(0.021)	B (0.007)	N (0.000)	D (1.000)	L (1.4)	D (-3.85)	D (0.359)	D (0.768)	22.3	D (0.058)
	14:23344816	c.659G>A	p.Cys220Tyr	5.73	D (0.0)	D (1.0)	D (0.999)	D (0.000)	D (1)	M (2.635)	T (-0.2)	D (0.127)	D (0.520)	25.6	D (0.093)
	14:23344989	c.832A>G	p.Thr278Ala	5.97	T (0.292)	P (0.911)	P (0.621)	D (0.000)	D (0.995)	L (0.975)	T (1.6)	T (-1.087)	T (0.067)	22.7	T (0.006)
	14:23345074	c.917G>A	p.Gly306Asp	5.97	T (0.055)	D (0.959)	P (0.6)	D (0.000)	D (0.997)	L (1.205)	D (-3.35)	D (0.601)	D (0.752)	23.5	D (0.048)
	14:23345931	c.1458G>C	p.Glu486Asp	5.91	D (0.036)	D (0.999)	D (0.963)	D (0.000)	D (1.000)	M (1.95)	D (-3.44)	D (0.841)	D (0.861)	28.1	D (0.039)
	14:23346254	c.1660C>T	p.Arg554*	5.23	NA	NA	NA	D (0.000)	D (1)	NA	NA	NA	NA	42	NA
	14:23346575	c.1981C>T	p.Arg661Cys	4.97	T (0.081)	D (0.999)	P (0.761)	D (0.000)	D (1.000)	L (1.1)	D (-3.18)	D (0.730)	D (0.805)	33	D (0.267)
	14:23341951	c.39C>T	p.Gly13=	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14:23344572	c.415A>G	p.Met139Val	2.05	T (0.302)	B (0.002)	B (0.003)	N (0.665)	N (1.000)	N (-0.625)	D (-2.19)	T (-1.010)	T (0.026)	3.321	NA
	14:23344848	c.691C>T	p.Arg231Trp	3.79	D (0.005)	D (1.0)	D (0.981)	D (0.000)	D (0.936)	M (2.085)	T (2.14)	T (-1.068)	T (0.032)	33	NA
	14:23345048	c.891C>T	p.Gly297=	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14:23345129	c.972C>T	p.Gly324=	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14:23345133	c.976G>A	p.Gly326Ser	4.07	T (0.584)	B (0.006)	B (0.019)	N (0.009)	D (0.861)	N (0.205)	D (-3.16)	T (-0.950)	T (0.115)	16.85	T (0.007)
	14:23346025	c.1552G>A	p.Asp518Asn	5.24	D (0.011)	D (0.993)	P (0.702)	D (0.000)	D (0.987)	L (1.39)	D (-3.11)	D (0.428)	D (0.645)	25.2	NA
	14:23346279	c.1685G>A	p.Arg562His	5.23	T (0.68)	D (0.999)	D(0.972)	D (0.000)	D (1.000)	M (2.2)	D (-3.37)	D (0.451)	D (0.772)	26.6	NA
	14:23346517	c.1923G>A	p.Leu641=	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14:23346682	c.2088G>C	p.Leu696=	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

GERP: Genomic Evolutionary Rate Profiling, SIFT: Sorting Intolerant From Tolerant, PolyPhen2 HDIV: Polymorphism Phenotyping version 2 human diversity, PolyPhen2 HVAR: Polymorphism Phenotyping version 2 human variation, LRT: Likelihood Ratio Test, FATHMM: Functional Analysis Through Hidden Markov Models, SVM: Support Vector Machine, LR: Logistic Regression, CADD: Combined Annotation Dependent Depletion, M-CAP: Mendelian Clinically Applicable Pathogenicity, T: tolerated, D: damaging/disease causing, B: benign, N: polymorphism/neutral, P: polymorphism automatic, L: low, M: medium, NA: not available. Transcript references: NM_014045-4. ANNOVAR (version 2018Apr16).

Supplementary Table 3: Other *LRP10* variants which did not fulfill our criteria for possible pathogenicity.

	Patient(s)	Genomic position	Nucleotide change	Amino acid change	Exon	Coding effect	dbSNP 142 accession number	Allele frequency GnomAD (alleles)	Functional predictions: pathogenic (total)	Splicing predictions: deleterious (total)
Discovery cohort	3-7	14:23341951	c.39C>T	p.Gly13=	2	synonymous	rs34294471	2.7% (7725)	NA	n.a.
	8	14:23345195	c.1038 C>T	p.Asp346=	5	synonymous	rs201657631	0.007% (20)	NA	n.a.
	9,10	14:23346279	c.1685G>A	p.Arg562His	7	missense	rs142153001	0.7% (1974)	9/10	n.a.
	11	14:23346630	c.2036A>C	p.His679Pro	7	missense	rs149685154	0.02% (52)	4/10	n.a.
Validation cohort	9-18	14:23341951	c.39C>T	p.Gly13=	2	synonymous	rs34294471	2.7% (7725)	NA	n.a.
	19, 20	14:23344572	c.415A>G	p.Met139Val	5	missense	rs28534929	0.7% (1974)	1/11	0/4
	21	14:23344848	c.691C>T	p.Arg231Trp	5	missense	rs35043211	0.2% (663)	7/11	n.a.
	22, 23	14:23345048	c.891C>T	p.Gly297=	5	synonymous	rs768801096	0.03% (94)	NA	n.a.
	24, 25	14:23345129	c.972C>T	p.Gly324=	5	synonymous	rs750833995	0.002% (5)	NA	n.a.
	26, 27	14:23345133	c.976G>A	p.Gly326Ser	5	missense	rs138170865	0.2% (450)	3/11	n.a.
	28	14:23346025	c.1552G>A	p.Asp518Asn	6	missense	rs74357167	0.4% (1053)	9/10	0/4
	29-40	14:23346279	c.1685G>A	p.Arg562His	7	missense	rs142153001	0.7% (1974)	9/10	n.a.
	41	14:23346517	c.1923G>A	p.Leu641=	7	synonymous	rs371430508	0.02% (60)	NA	n.a.
	42	14:23346682	c.2088G>C	p.Leu696=	7	synonymous	rs148391884	0.002% (6)	NA	1/4

Only variants in exons or at the exon-intron boundary (-10/+10) are displayed. The Genome Reference Consortium Human Build 37 (hg19) and NM_014045-4 LRP10 transcript were used. GnomAD: Genome Aggregation Database, NA.: not available, n.a.: not applicable. Splicing prediction programs: SSF, MaxEnt, NNSPLICE, Gene Splicer.

Supplementary Table S4: Possibly pathogenic variants in known genes causing parkinsonism or dementia.

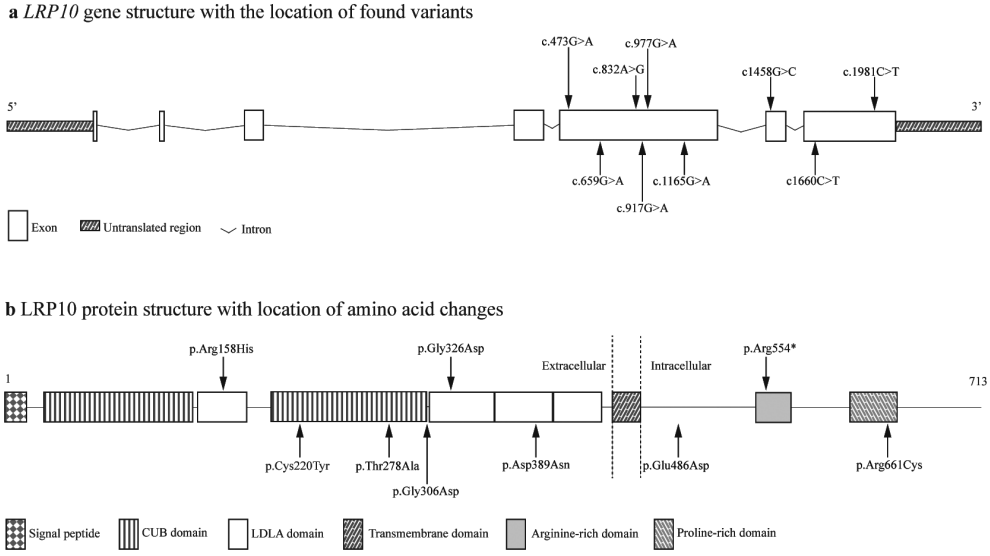
Patient	Gene	Genomic position	Nucleotide change	Amino acid change	Exon	Coding effect	dbSNP 142 accession number	Allele frequency GnomAD (alleles)	Functional predictions: pathogenic (total)
1	*VPS13C*	15:62211490	c.7636C>T	p.Gln2546*	58	stopgain	.	.	n.a.

Only variants in exons or at the exon-intron boundary (-10/+10) are displayed. The Genome Reference Consortium Human Build 37 (hg19) and NM_020821 VPS13C transcript were used; GnomAD: Genome Aggregation Database, n.a..: not applicable.

Supplementary Table 5: Overview of identified variants in current and previous studies.

	Genomic position	Nucleotide change	Amino acid change	Study	Present in number of patients (diagnosis)	Present in number of controls
Discovery cohort	14:23345322	c.1165G>A	p.Asp389Asn	Current	1/950 (PSP)	0/645
	14:23345134	c.977G>A	p.Gly326Asp	Current	1/950 (PSP)	0/645
				Pihlström et al., 2018[7]	1/264 (MSA)	0/462
Validation cohort	14:23344630	c.473G>A	p.Arg158His	Current	1/950 (PSP)	0/645
				Kia et al., 2018[8]	0/2835 (PD)	1/5343
	14:23344816	c.659G>A	p.Cys220Tyr	Current	1/950 (PSP)	0/645
	14:23344989	c.832A>G	p.Thr278Ala	Current	1/950 (PSP)	0/645
	14:23345074	c.917G>A	p.Gly306Asp	Current	2/950 (PSP)	0/645
				Kia et al., 2018[8]	2/2835 (PD)	1/5343
	14:23345931	c.1458G>C	p.Glu486Asp	Current	1/950 (PSP)	0/645
				Kia et al., 2018[8]	3/2835 (PD) and 1/111 (DLB)	0/5343 and 0/233
	14:23346254	c.1660C>T	p.Arg554*	Current	1/950 (PSP)	0/645
				Guerreiro et al., 2018[9]	0/1040 (DLB)	1/1422
	14:23346575	c.1981C>T	p.Arg661Cys	Current	1/950 (PSP)	0/645
				Pihlström et al., 2018[7]	0/264 (MSA)	1/462
				Kia et al., 2018[8]	0/2835 (PD)	1/5343

PSP: progressive supranuclear palsy, MSA: multiple system atrophy, PD: Parkinson's disease, DLB: dementia with Lewy bodies.



Supplementary Figure 1:
(a) *LRP10* gene structure with the location of found variants. (b) LRP10 protein structure with the location of amino acid changes.

Supplementary References

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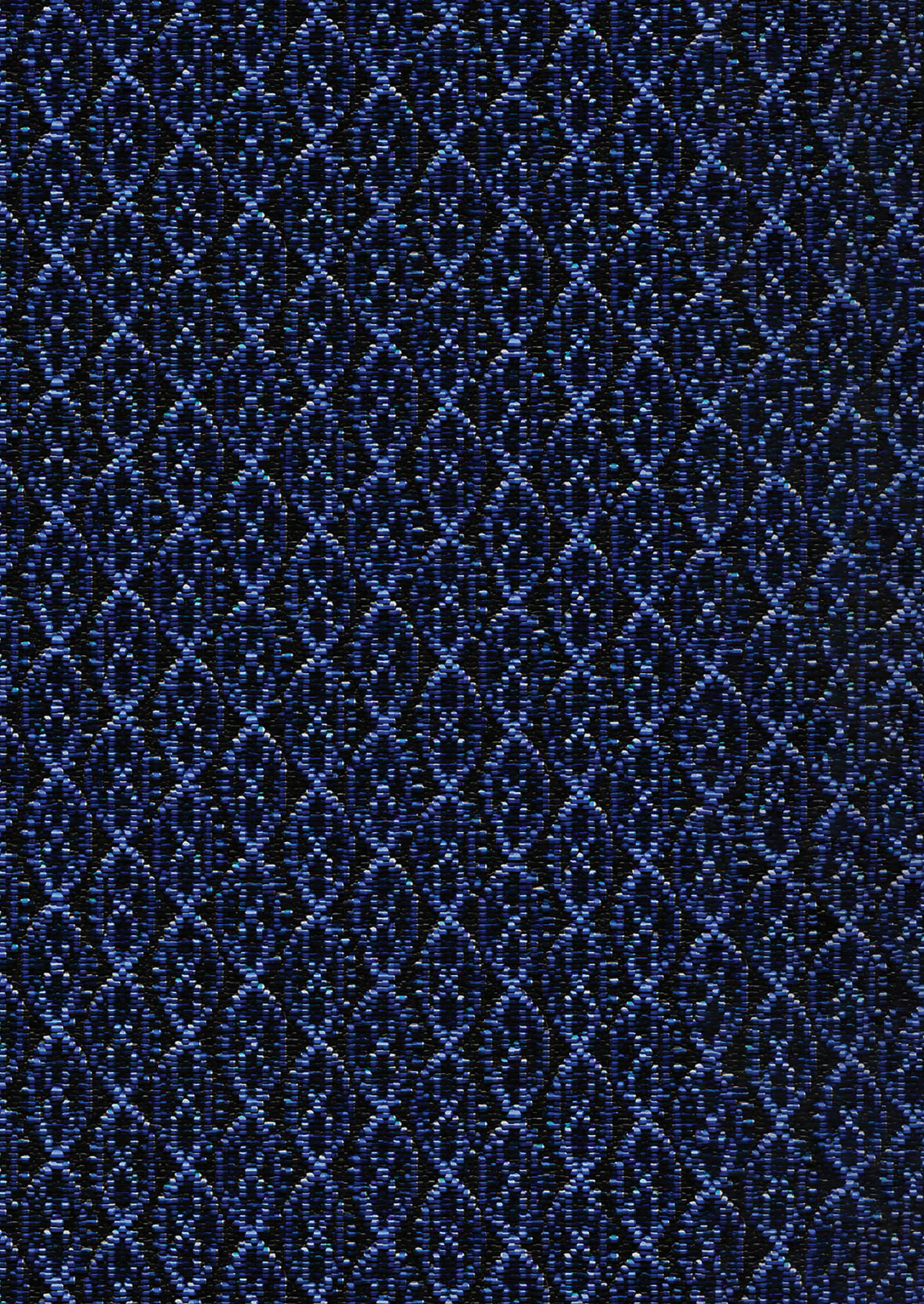

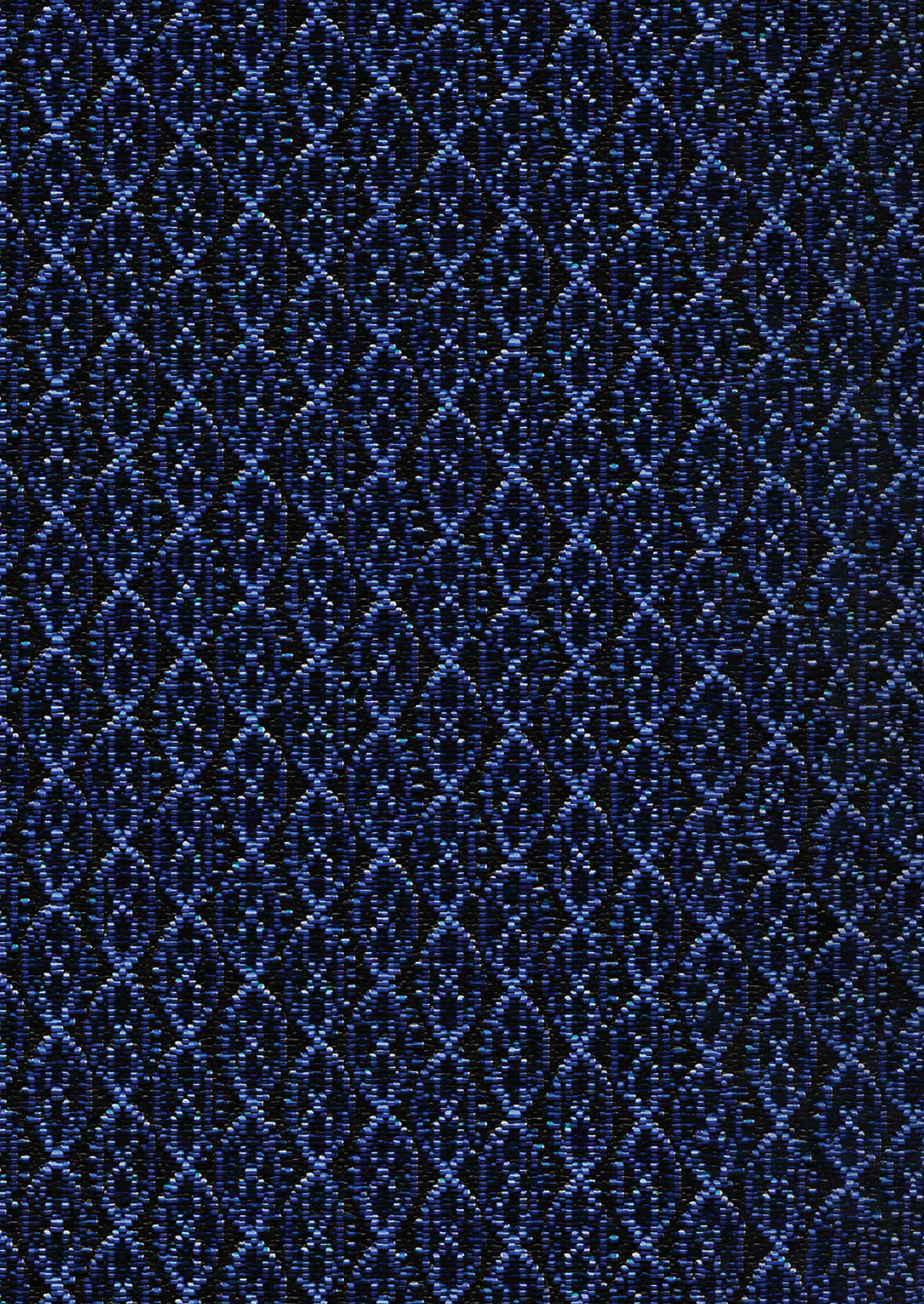

Part 6

General discussion

Dementia with Lewy bodies (DLB) is a common neurodegenerative disease of the elderly[1] and is characterized by progressive cognitive decline, parkinsonism, visual hallucinations, fluctuating cognition, and REM-sleep behavior disorders.[2] Clinical features, pathological hallmarks and genetic factors of DLB overlap with those of Alzheimer's disease (AD) and Parkinson's disease (PD).[1] Genetic factors seem to play a considerable role in DLB, as the heritable component of DLB has been estimated to be approximately 60%.[3,4] However, few genetic studies, often with small sample sizes and candidate gene-based (biased) approaches, have been undertaken in DLB (as described in our review in **Chapter 1.2)**.[5] The first genome wide association (GWA) studies of DLB have only been performed recently, and whole exome sequencing (WES) studies have not been published yet. The paucity of genetic studies in DLB could be due to several reasons. Firstly, DLB has only been recognized as a separate disease since 1984. Up until that time, DLB was referred to as the Lewy body variant of AD or grouped under the umbrella term Lewy body diseases, which is comprised of PD, PD dementia (PDD) and DLB. Secondly, the accuracy of the clinical criteria for DLB is relatively low and many DLB patients are misdiagnosed or overlooked.[6] This could have led to small and heterogeneous study groups. Thirdly, DLB patients with multiple relatives suffering from DLB, AD, or PD have only rarely been recognized, which hampers the identification of rare, highly-penetrant genetic variants in this disease. As a consequence, only several genetic factors (the *APOE* ε4 allele, specific disease-associated variants in *GBA* and *SNCA*) have been associated with DLB up until now. These genetic factors seem to explain only a small percentage of the total heritability[4], which suggests that many genetic factors still remain to be discovered.

This thesis describes our findings on the familial aggregation in DLB, the role for variants in known AD and PD genes in DLB, and the search for novel genes associated with DLB. This Part begins with a discussion of these findings in light of current literature. Subsequently, an overview is provided on how the identified genes may play a role in the molecular mechanisms leading to DLB, and on how these genes may aid in developing biomarkers and disease-modifying treatment options in the future. Hereafter, the strengths and limitations of the studies contained in this thesis are discussed. Finally, suggestions for future study directions are provided.

Familial aggregation

The precise frequencies of DLB patients with relatives with DLB, AD, or PD are currently unknown. Nonetheless, it has been noted that AD and PD occur more frequently in relatives of DLB patients than in relatives of controls[7,8], and that DLB has a higher prevalence in relatives of DLB patients than in relatives of AD patients.[9] Families with multiple members with DLB, compatible with an autosomal dominant pattern of inheritance, have rarely been described. Mutations in *SNCA, LRRK2, PSEN1, PSEN2,* and *APP* have been identified in a handful of DLB patients with relatives with DLB, PD, or dementia.[5] These genes have

previously been associated with PD (*SNCA, LRRK2*) or AD (*PSEN1, PSEN2, APP*). Genetic factors associated with DLB, PD, and AD seem, therefore, to overlap, but little is known about the magnitude of this overlap and its effect on different phenotypes.

In this thesis, we studied possible differences in phenotype between DLB patients with relatives with dementia or PD (familial patients) and DLB patients without relatives with these diseases (sporadic patients) (**Chapter 2.1**) and between DLB patients with and without known, well-established genetic risk factors (**Chapter 3.1**). In **Chapter 2.1** we showed that familial DLB patients have a shorter survival than sporadic DLB patients. We also found a higher percentage of familial DLB patients with elevated AD biomarkers in their cerebrospinal fluid (CSF) compared to sporadic DLB patients. This suggests that genetic factors influence the course of the disease, possibly by affecting the amount of concomitant AD pathology. The exploratory study elaborated in **Chapter 3.1** suggested that DLB patients with the *APOE* ε4 allele more often have a positive family history of AD or dementia than non-carriers. Furthermore, a family history of PD or PDD was present at a greater frequency in DLB patients with disease-associated *GBA* variants than in DLB patients with the *APOE* ε4 allele. Interestingly, we also found that DLB patients with relatives with PD more often show parkinsonism as first symptom than DLB patients with a family history of dementia (**Chapter 2.1**).

These findings suggest that different subgroups may be identified within the DLB population: patients with a phenotype which more closely resembles PD, with possibly underlying PD-associated genetic factors, and patients with a phenotype which more closely resembles AD, with possibly underlying AD-associated genetic factors and mixed neuropathology.

In the next paragraph, I will discuss which well-established genes are associated with DLB and how they overlap with genes associated with AD and PD.

Well-established genes associated with DLB

There are three well-established genes associated with DLB: *APOE*, *GBA* and *SNCA*. Variants in these genes have been associated with DLB in multiple different candidate gene studies (**Chapter 3.1** and **Chapter 3.2**)[10,11] and in two recent GWA studies.[12,13] The *APOE* ε4 allele is the most common risk factor for DLB.[10,12-14] An odds ratio of 2.9 has been reported for carriers of one *APOE* ε4 allele and of 5.9 for carriers of two *APOE* ε4 alleles for developing DLB.[15] DLB patients with an *APOE* ε4 allele have a shorter survival than DLB patients without this allele.[16-18] Conversely, *APOE* ε2 allele carriers have a reduced risk of developing DLB compared to controls.[15] For *GBA*, the risk of developing DLB is dependent on the specific disease-associated variant.[19,20] An odds ratio of 8 for developing DLB has been reported for carriers of disease-associated *GBA* variants in one study.[11] DLB patients with disease-associated *GBA* variants display an earlier age of onset and earlier age of death than patients without these variants.[11,14,21,22] Disease-associated variants in *SNCA* can modify

the risk of developing DLB, and can be, very rarely, the cause of DLB. Families with *SNCA* mutations show a large heterogeneity in diagnosis (DLB, PDD, PD), age of onset and disease progression.[23-26] The *SNCA* locus has been found and replicated in a GWA study of DLB, including 1743 cases and 4454 controls.[12] However, the exact *SNCA* variants which increase the risk of developing DLB remain controversial. Furthermore, the magnitude of this risk and the effect of these genetic variants on phenotype remain unexplored.

The three well-established genes which are associated with DLB (*APOE*, *GBA* and *SNCA*), also play a role in the development of AD or PD. The *APOE* ε4 allele is the most important genetic risk factor for AD, and disease-associated *GBA* variants increase the risk of developing PD and cognitive decline in PD.[27,28] Rare causal variants and common disease-associated variants in *SNCA* have repeatedly been identified in PD patients.[29-31] Intriguingly, different association profiles in *SNCA* have been found for PD and DLB.[12] The single nucleotide polymorphism (SNP) showing the highest association with DLB for the *SNCA* locus (rs7681440) was not significantly associated with PD. Equally, the SNP showing the highest association with PD for the *SNCA* locus (rs356182) was not significantly associated with DLB. Thus, it may be hypothesized that these differences lead to different gene expression profiles and different molecular mechanisms between DLB and PD.

Besides *APOE*, *GBA* and *SNCA*, other genetic loci associated with AD or PD, have not been identified by GWA studies in DLB.[12,13] Furthermore, a recent study has shown that known AD and PD genetic risk loci only explain a very small percentage (~1.3% and ~0.4%, respectively) of the phenotypic variance in DLB[4].

To summarize, some genetic factors are shared between DLB and AD or PD, which may lead to similar molecular mechanisms and possible clinical features; however, the majority of genetic factors associated with DLB might be independent of those associated with AD and PD and still have to be discovered.

Novel genes associated with DLB

LRP10

Rare variants in the *low-density lipoprotein receptor related protein 10* gene (*LRP10)* were reported for the first time in familial forms of PD, PDD, and DLB by Quadri et al., in 2018.[32] In this study, genome-wide linkage analysis in a family with dominantly inherited PD (10 affected members) was performed to map the disease locus to a region in chromosome 14. Subsequently, WES identified a single nucleotide substitution in *LRP10,* leading to a missense change (Gly603Arg) as the most likely disease-causing variant. This was followed by the sequencing of the whole *LRP10* coding region in 660 unrelated PD, PDD, and DLB patients and 645 controls, and by the sequencing of specific possibly pathogenic *LRP10* variants in additional series of 1448 PD patients and 811 controls. Eight patients and one control from the first series and three patients and no controls from the second series were identified with a possibly pathogenic *LRP10* variant. Furthermore, the variant present in the

index cases was also present in 9 out of 10 additional affected relatives with DNA available for testing. Three patients carrying one of the possibly pathogenic *LRP10* variants could be studied post-mortem and they all had a severe burden of Lewy pathology. Functional studies in cell cultures showed that the *LRP10* variants affected mRNA stability, protein stability or protein localization.

Several studies have tried to replicate these initial findings. Five studies failed to find evidence for an association between *LRP10* variants and PD, PDD, DLB, progressive supranuclear palsy (PSP), multiple system atrophy (MSA), or frontotemporal dementia (FTD).[33-37] However, the absence of evidence in these studies does not allow to conclude for the absence of association. There are several reasons why these replication studies might have failed to detect a role of *LRP10* in these diseases.

First, and most importantly, case-control association studies are not a powerful strategy to detect or replicate the role of rare, highly-penetrant variants in the disease etiology, unless only large series of patients with familial forms of the disease are considered. In some of the above-mentioned studies, the power to detect rare variants was not optimal because of limited sample sizes. In the original paper, the research sample was comprised of predominantly familial patients with late-onset disease. Three of the replication studies included sporadic patients[33-35], and one analyzed patients with young-onset disease.[33] Second, possibly pathogenic *LRP10* variants may be population specific. The ethnicity of the patients in two of the replication studies were substantially different than the original study.[33,37] Third, the control groups may have introduced bias, as they also included relatively young, unaffected participants who could still develop a late-onset neurodegenerative disease like PD or DLB.[33,34] Furthermore, some control groups also contained participants who had not been clinically examined.[34] Fourth, almost all replication studies adopted more relaxed filtering criteria for possibly pathogenic variants than those used in the original paper. This may have biased the results, as truly pathogenic and benign variants might have been grouped together. It is also possible that only some, specific variants are truly pathogenic and that these variants are more frequently found in patients than in controls. These variants will not be recognized as such with the used analysis methods.

We (**Chapter 4.1, Chapter 4.2** and **Chapter 4.3**) and three other research groups[36,38,39] found further evidence for an involvement of *LRP10* in PD, dementia with parkinsonism without Lewy pathology, dementia with Lewy pathology, and PSP. Of particular interest is one of the variants, p.Tyr307Asn, as this variant was identified in the original study in a familial PDD patient from Italy, and subsequently in a mother and daughter with PD from France, and then in a PD and PDD patient with a shared ancestor from the Netherlands (**Chapter 4.1**).[36,40,41] Considering all these studies together, the frequency of this specific variant seems significantly higher in familial PD and PDD patients in comparison to large control groups such as the Genome Aggregation Database (total 15 alleles in 251,270).[41] The p.Tyr307Asn variant has also been shown in functional studies to decrease LRP10 protein stability.[40] However, it should be noted that the p.Tyr307Asn variant was not found in another

two relatives affected with PD in the same French family mentioned earlier, and they may represent phenocopies. In our study of patients with dementia and parkinsonism without Lewy pathology, and patients with dementia and Lewy pathology, we identified three rare and possibly pathogenic *LRP10* variants (**Chapter 4.2**). Interestingly, in previous studies, all these three variants were identified in additional unrelated patients with PDD, PD, MSA and in a single unaffected person (not neurologically examined)[33,35,40,41]. Interestingly, no Lewy pathology was observed in one of our patients with AD and parkinsonism carrying a possibly pathogenic *LRP10* variant. Our PSP study (**Chapter 4.3**) showed that an increased fraction of these patients carries a possibly pathogenic *LRP10* variant compared to controls. These results suggest that *LRP10* variants may be associated with several neurodegenerative diseases with different underlying neuropathologies.

In conclusion, our studies provide additional evidence for a role of *LRP10* in PD, DLB, PSP and possibly AD. Nonetheless, there is still no firm consensus about the role of *LRP10* in these neurodegenerative diseases, and additional robust and independent replication is warranted, preferably from studies in large families with co-segregation of the variant in multiple affected members.

A multimodal approach to find novel genes

In a parallel effort, we tried to find novel genes for DLB by combining WES data and CSF proteomics data. We described the results of the CSF proteomics analysis in **Chapter 5.1**, and the combination of the WES data and CSF proteomics data in the **Appendix to Chapter 5.1**. Besides the use of CSF proteomics to find novel genes for DLB, this analysis is also very interesting to find novel fluid biomarkers for the disease. In **Chapter 5.1** we identified and validated six novel candidate CSF biomarkers (VGF, SCG2, NPTX2, PDYN and PCSK1N) for DLB. These proteins are, amongst others, involved in neurotransmitter release, the packaging of neuropeptides in secretory vesicles and the formation of new excitatory synapses[42-44], and may be markers of synaptic dysfunction. Synaptic dysfunction is observed in many neurodegenerative diseases, which led to the question of whether the identified biomarkers are specific for DLB. We analyzed these markers, therefore, in cohorts of AD, PD and FTD patients, which showed that the CSF levels of PDYN, SCG2 and VGF are significantly lower in DLB than in the other groups. To improve the discrimination of DLB and the other neurodegenerative diseases, machine learning was used to identify the most optimal biomarker panel. This resulted in a panel of PDYN, SCG2 and VGF with an accuracy of 0.82, specificity of 0.83 and sensitivity of 0.69. Further studies in larger and independent cohorts are needed to investigate the possible clinical usefulness of these markers.

In the pilot study described in the **Appendix to Chapter 5.1** we combined these CSF proteomics data and WES data to identify novel candidate genes associated with DLB. The hypothesis behind this approach is that a pathogenic genetic variant will lead to a differential expression of the corresponding protein in the CSF. Our pilot study focused on DLB patients with relatives with dementia or PD to increase the likelihood of detecting disease-associated

findings. Although we identified an overlap between eight genes and proteins, the evidence of an association with DLB is limited. We discuss the limitations of this study and suggestions for improving such study designs in the future in the 'Strenghts and limitations' and 'Future study directions' sections.

Molecular mechanisms underlying DLB

The identification of genes associated with DLB might contribute to a better understanding of the underlying molecular disease mechanisms. Multiple molecular pathways are likely implicated in the development of neurodegeneration due to primary defects in *APOE*, *GBA*, *SNCA,* and *LRP10,* but many of these molecular mechanisms are not fully elucidated. Here, I describe the key molecular mechanisms in which these genes have been implicated up until now.

APOE encodes the protein Apolipoprotein E, which plays a role in lipid transport, synaptic integrity, neuroplasticity, neuroinflammation, tau phosphorylation, glucose metabolism, and cerebrovascular function.[45] APOE has been shown to affect amyloid-β clearance and aggregation leading to AD pathology. Several studies have indicated an APOE isoform-dependent effect, in which the *APOE* ε4 isoform is less efficient in amyloid-β trafficking and degradation than the *APOE* ε3 isoform.[46,47] It has also been demonstrated that the *APOE* ε4 isoform promotes the formation of amyloid-β fibrils and amyloid-β production.[45] At autopsy, the majority of patients with DLB have both Lewy as well as AD pathology.[48,49] Interestingly, one study has indicated that the *APOE* ε4 allele is not only associated with DLB with AD pathology, but also with DLB without AD pathology.[10] This suggests that the *APOE* ε4 allele also plays a role in amyloid-β independent mechanisms leading to DLB.

GBA encodes the protein Glucocerebrosidase (GCase), a lysosomal enzyme. GCase dysfunction has been shown to be related to an impaired autophagy-lysosomal system, increased endoplasmic stress, mitochondrial dysfunction and α-synuclein aggregation.[50-52] GCase hydrolyses glucosylceramide (GlcCer) and glucosylsphingosine (GLSph). Mutations in *GBA* result in a reduction of the enzymatic function of GCase, which leads to the accumulation of GlcCer and GLSph in lysosomes. This could lead to autophagy-lysosomal and mitochondrial dysfunction, and the accumulation of α-synuclein. Conversely, α-synuclein accumulation may result in the retention of GCase in the endoplasmic reticulum, leading to impaired GCase trafficking to the lysosome and to endoplasmic reticulum stress.[53] This indicates that GCase and α-synuclein might be part of a bidirectional pathogenic loop.[54]

SNCA encodes the protein α-synuclein, one of the major constituents of Lewy bodies and neurites.[55] α-Synuclein has been implicated in synaptic plasticity, neurotransmitter release, and synaptic vesicle pool maintenance.[56-58] Disease-causing mutations in *SNCA* seem to increase the susceptibility of the protein to aggregate.[59]

LRP10 encodes a plasma membrane receptor (LRP10) whose function remains largely elusive. It has been suggested that LRP10 plays a role in the metabolism of

APOE lipoproteins[60] and APP trafficking and processing, which may influence amyloid-β homeostasis.[61] Interestingly, a decreased expression of LRP10 in the brains of AD patients compared to controls has been reported, even if caution is warranted here, as the specificity of the antibody used to detect the LRP10 protein in the human brain tissue was not adequately demonstrated.[61] LRP10 may also interact with VPS35, a component of the retromer complex and the product of another PD-causing gene, and with GGA proteins, which have been shown to promote α-synuclein aggregation.[62-64] Further research is warranted to study if LRP10 expression is also modified in the brains of PD or DLB patients, and to elucidate the interactions between LRP10, APOE, APP, and α-synuclein aggregation.

In conclusion, the current evidence suggest that *APOE*, *GBA*, *SNCA* and *LRP10* may be involved in similar or interacting neurobiological pathways driving Lewy and AD pathology. Further studies are needed to gain more information about the different molecular mechanisms leading to DLB. This is important for future development of biomarkers and disease-modifying treatments.

Future applications

Biomarkers

The identification of genes associated with DLB could lead to the implementation of these genes or their derivatives (e.g. encoded or interacting proteins in CSF or blood) as biomarkers in clinical practice. Biomarkers can be useful in 1) the diagnostic process, 2) the prognostic process, 3) the identification of at-risk persons, and 4) personalized medicine.

Diagnostic biomarkers which improve the accuracy of the DLB diagnosis are highly needed, as the accuracy of current clinical criteria for DLB is still relatively low.[6] GCase and α-synuclein in blood or CSF are interesting candidate biomarkers, which could possibly be used for this purpose in the future. Several studies have indicated that CSF levels of GCase are not only decreased in PD patients with disease-associated *GBA* variants, but also in PD patients without these genetic variants.[65,66] GCase has also been found at decreased levels in the blood of sporadic PD patients as compared to controls. Furthermore, GCase correlated with α-synuclein levels in the brain of PD patients, possibly due to the effects of α-synuclein on GCase.[54] GCase CSF levels are also decreased in DLB patients with disease-associated *GBA* variants compared to controls.[67] However, it is currently unknown whether GCase CSF levels are also downregulated in DLB patients without disease-associated genetic variants, and in the blood of DLB patients. In addition, CSF levels of total α-synuclein have been found to be decreased in DLB patients compared to AD patients and controls, but not in comparison to PD patients.[68,69] Conversely, CSF levels of oligomeric α-synuclein are increased in DLB patients compared to AD patients and controls, but not compared to PD patients.[13] These findings suggest that levels of GCase, total α-synuclein, and oligomeric α-synuclein may be useful in differentiating DLB patients from AD patients and controls.

Prognostic biomarkers based on genetic factors are not yet used in clinical practice. The

main reason for this is that the disease course of carriers of a specific genetic variant is very variable. Indeed, even in families with *SNCA* mutations the disease course is largely unpredictable.[23,26] However, several differences between genetic variant carriers and non-carriers have been found at group level: DLB patients carrying the *APOE* ε4 allele have a shorter survival than non-carriers[16-18], and DLB patients carrying disease-associated *GBA* variants have an earlier onset and an earlier death than non-carriers.[11,14,21,22] Our study described in **Chapter 2.1** also showed a different disease course between familial and sporadic DLB patients. This provides further evidence that genetic factors can be useful to estimate the prognosis in different patient groups. Further studies are needed to clarify whether genetic factors can also be used to generate individual predictions of disease course.

In DLB, the prediction of disease development and the selection of at-risk persons for medical trials by using biomarkers, is not yet a reality. One exception to this is in the testing of causal mutations in *SNCA* in families with multiple patients with DLB, PDD, and PD. The results of this test could identify individuals who have a high risk of developing DLB, PDD or PD. *LRP10* genetic testing in clinical practice awaits conclusive replication of the role of variants in this gene in independent studies. Furthermore, current evidence points to a reduced penetrance for at least some *LRP10* variants, which limits the predictive value for an individual person. Genetic testing for risk factors of DLB, such as the *APOE* ε4 allele and disease-associated *GBA* variants, is also not currently recommended for clinical purposes. Results of such testing explain only a very small fraction of the total prediction, which is not informative by itself. However, polygenic risk scores, in which the combined risk of multiple genetic factors is considered, may ultimately be useful to predict the risk of disease development. The general belief is that the pathological process that leads to neurodegenerative diseases can only be reversed or prevented when medication is taken in the early pre-symptomatic phase of the disease. The identification of at-risk persons is, therefore, of utmost importance to detect alterations in molecular mechanisms and apply novel treatment options, before the onset of clinical symptoms. Furthermore, genetic status may be crucially important if treatment options are only found to be effective in the carriers of specific genetic variants.

Treatment

The ultimate goal of studying genes associated with DLB is to increase our understanding of the disease pathogenesis, which could eventually aid in the development of disease-modifying treatments. Treatment options targeting genes or related molecular mechanisms associated with DLB may not only be beneficial to patients with disease-associated genetic variants, but to all DLB patients. Unfortunately, no clinical trials have yet been performed or are currently underway in DLB specifically, targeting well-established genes associated with DLB or related molecular mechanisms. However, related clinical trials are presently being performed on patients with AD, patients with mild cognitive impairment (MCI), PD and PDD concerning *APOE*, *GBA,* and *SNCA*.

Recently, two phase 1 clinical trials have commenced targeting the *APOE* gene in patients with AD and MCI due to AD pathology. The target of one of these trials is to assess the safety and toxicity of the intracisternal administration of adeno-associated viral vectors (AAVs) expressing the cDNA coding *APOE* ε2 in patients with two *APOE* ε4 alleles (ClinicalTrials.gov identifier NCT03634007). In the other trial, the safety of plasma infusion from young adults with two *APOE* ε3 alleles to MCI patients with two *APOE* ε4 alleles will be determined (ClinicalTrials.gov identifier NCT03887741). The rationale behind these studies is that the detrimental effects of carrying two *APOE* ε4 alleles will be diminished.[70]

At present, several clinical trials are being performed in PD and PDD, which target *GBA* and associated molecular mechanisms. One such phase 2 trial in PD patients with disease-associated *GBA* variants assesses the drug dynamics, efficacy, and safety of Venglustat, a GlcCer synthase inhibitor (ClinicalTrials.gov identifier NCT02906020).[71] Two other phase 2 clinical trials, one in PD and one in PDD patients with disease-associated *GBA* variants, test the safety, tolerability, pharmacodynamics, and clinical effects of Ambroxol (ClinicalTrials.gov identifier NCT02941822 and NCT02914366). Ambroxol is a small molecular chaperone, which aids physiological posttranslational folding of mutant GCase, resulting in the upregulation of GCase trafficking to the lysosome.[72] Another potential disease-modifying treatment option for disease-associated *GBA* carriers is gene therapy, in which AAVs coding for *GBA* increases GCase activity. A phase 1/2a trial has recently begun to evaluate the safety of intracisternal administration of AAVs in PD patients with at least one *GBA* disease-associated variant (ClinicalTrials.gov identifier NCT04127578). The results of these trials are still pending.

For *SNCA*, different compounds have been examined with the aim to decrease α-synuclein aggregation, increase α-synuclein degradation, or decrease extracellular α-synuclein. For instance, one phase 2 trial concerned the small molecule, Nilotinib, an inhibitor of c-Abl tyrosine kinase, which is involved in many essential cellular processes, such as cell growth, neurogenesis and neurite extension.[73] The non-blinded, non-placebo-controlled safety trial with the aforementioned drug showed some improvement in motor function of DLB patients and PDD patients.[74] Different trials utilizing both passive and active immunotherapies to reduce extracellular α-synuclein are currently ongoing. Other promising therapies involving *SNCA* involve the reduction of the production of α-synuclein by gene silencing mechanisms using antisense oligonucleotide therapy and altering histone acetylation of the promotor and enhancer regions of *SNCA*.[71]

Positive results originating from these trials will stimulate the development and testing of treatment options in DLB. Personalized medicine, in which a combination of different drugs targets different genes and molecular mechanisms based on the genetic profile of the patient, is a very promising approach in the future.

Strengths and limitations of the studies described in this thesis

The first strength of the described studies is the relatively large sample size of the studies in **Part 2** and **Part 4**. We collaborated with several research groups to obtain such a large quantity of patients. It should be noted, nonetheless, that the sample sizes of the patient groups in **Part 3** and **Part 5** were relatively small. However, these study groups were carefully selected subgroups of patients, which had never been studied in such a comprehensive or novel manner before. As the accuracy of the clinical diagnosis of DLB remains suboptimal[6], we did not only study clinical diagnosed DLB patients, but also pathologically confirmed DLB patients. We have found similar results in clinical as well as in pathologically confirmed patients (**Part 3**), which underpins the reliability of our findings. Other strengths include the use of Sanger sequencing for the initial analyses or validation of genetic variants in **Part 3** and **Part 4**, and the replication of our findings in independent cohorts in **Chapter 4.3** and **Chapter 5.1**.

The marked limitation of the described studies is that bias may have been introduced by relying on retrospective clinical data. Only a portion of the data on family history was obtained by means of a structured patient/caregiver interview, and patient/caregivers were often not able to provide accurate information on which diagnoses were made in their relatives. A bias in family history may also be introduced by relatives who died before symptom onset, and by asymptomatic relatives at time of inclusion who developed the disease later in life (**Part 2** and **Part 3**). Another limitation is that co-segregation studies could only be performed in a small percentage of patients with relatives with neurodegenerative diseases (**Part 3, Part 4,** and **Appendix to Chapter 5.1)**. The main reason for this is that often no DNA was available from deceased relatives and that younger relatives were not (yet) affected by disease. Limitations of the CSF proteomics study (**Part 5**) are, amongst others, that proteins could have been missed due to a bias towards the identification of peptides with higher concentrations in mass spectrometry studies, and that the control group consisted of individuals with subjective cognitive complaints. Furthermore, a bias could have been introduced by comparing the levels of the candidate biomarkers between DLB patients and the patients with other neurodegenerative diseases for which different control groups and techniques were used. The limitations of the pilot study (**Appendix to Chapter 5.1**) are, amongst others, that it is possible that CSF protein levels may not be altered at all by pathogenic genetic defects, and that we did not use an adjusted reference protein database, which may have led to the missing of altered peptides.

Future study directions

Although this thesis has contributed to the knowledge of the genetics of DLB, many genetic factors associated with the disease remain unknown. In general, future genetic studies will benefit from a more accurate clinical diagnosis, a better documentation of family history,

larger samples sizes and the use of novel research strategies and techniques.

In clinical practice, DLB remains markedly under-recognized. This could be due to unacquaintance of family doctors and specialists with the concept and the clinical criteria of DLB, resulting in a failure to ask and examine the patients for all the possible symptoms and signs of DLB.[1,75] Additionally, the accuracy of clinical diagnostic criteria for DLB is still relatively low and DLB is often misdiagnosed as AD.[6,76] Especially the sensitivity of the clinical criteria of DLB should increase to improve an early diagnosis. Enhancing these aspects of disease identification will ultimately lead to larger and more homogeneous study groups. Furthermore, it is important to estimate how often DLB patients have relatives with DLB or related disorders. This has yet to be understood and could alter our perception of the genetics of DLB, leading to more refined and powered genetic research into rare variants associated with DLB. Therefore, taking a careful and structured family history in DLB is essential and should be promoted among health care providers.

To find novel, common variants associated with DLB, samples sizes of GWA studies have to increase drastically. To date, the largest GWA study performed in DLB included 1743 patients and 4454 controls[12], which are relatively small numbers for a GWA study. The statistical power to find novel risk factors with low effect sizes is limited with these relatively low sample sizes. Larger GWA studies are necessary to find new genetic factors with a small to moderate effect size. In AD and PD, GWA studies reach sample sizes of around 15.000 patients and >40.000 controls. Such large cohorts are necessary to identify risk factors with effect sizes as low as 1.1 - 1.4.[30,77] GWA studies with more patients are, therefore, essential to find more risk factors with small effect sizes associated with DLB. National and international collaborations are essential in these efforts.

To find novel, rare variants associated with DLB different study approaches can be pursued. For instance, WES or whole genome sequencing (WGS) could be performed in large cohorts or in subgroups of patients in which the chances of finding rare variants is higher than in the total DLB population, such as families with multiple patients with DLB, PD(D), or dementia. Another possibility is to use a multimodal approach, such as the combination of genetics and proteomics, as shown in our pilot study (**Appendix to Chapter 5.1**). Future research combining genetics and proteomics with the aim to identify new genes associated with disease are promising if: 1) a strong family history is present, 2) DNA of multiple relatives is available, 3) a small set of possibly pathogenic genetic variants is obtained after genetic analysis, 4) tissue of interest is available in preferably >1 relative, and 5) the protein reference database is adjusted to recognize mutated peptides. The preferable tissue of interest when combining genetic and proteomic data in DLB might be neurons or brain inclusions. However, it should be considered that these data represent proteomic changes in late stage disease, which may not reflect protein differences due to a primary genetic defect. Other omics data, such as epigenomic, transcriptomics and metabolomic data, could also be used in multimodal approaches.

Novel common and rare variants identified by future GWA, WES and WGS studies will contribute to a higher percentage of explained heritability for DLB. Furthermore, a part of the

heritability will probably be explained by copy number variation (CNV) and epigenetics, such as enhancers and other regulatory elements, gene-gene interactions and gene-environment interactions. Unbiased studies into these domains are still in its infancy concerning DLB.

Conclusion

Despite the appreciation that DLB has a substantial heritable component, only few genetic studies have been performed. This thesis adds to the understanding of the genetics of DLB. First, it showed that DLB patients with relatives with dementia or PD have a different phenotype than DLB patients without a positive family history. Second, we confirmed that the *APOE* ε4 allele and disease-associated variants in *GBA* are important genetic factors associated with familial DLB and DLB with a rapidly progressive disease course. Third, we found more evidence that the recently identified *LRP10* gene is associated with PD, DLB, PSP and possibly AD. Fourth, we found several new candidate CSF biomarkers associated with DLB, which could potentially increase the accuracy of the DLB diagnosis.

Nonetheless, known genetic factors associated with DLB still explain only a small part of the total heritability of DLB. The majority of genetic factors associated with DLB still remain to be discovered. Large, homogeneous cohorts of DLB patients and very well-defined families with DLB and related disorders are needed to find new genetic variants associated with DLB. These genetic factors, in combination with environmental, epigenetic, and stochastic factors, will likely all play important roles to determine the resulting individual risk of developing DLB.[78]

The coming years promise to be exciting as many new genetic factors are likely to be discovered. These findings may lead to more accurate diagnosis, a better prediction of prognosis, and, ultimately the development of disease-modifying treatments.

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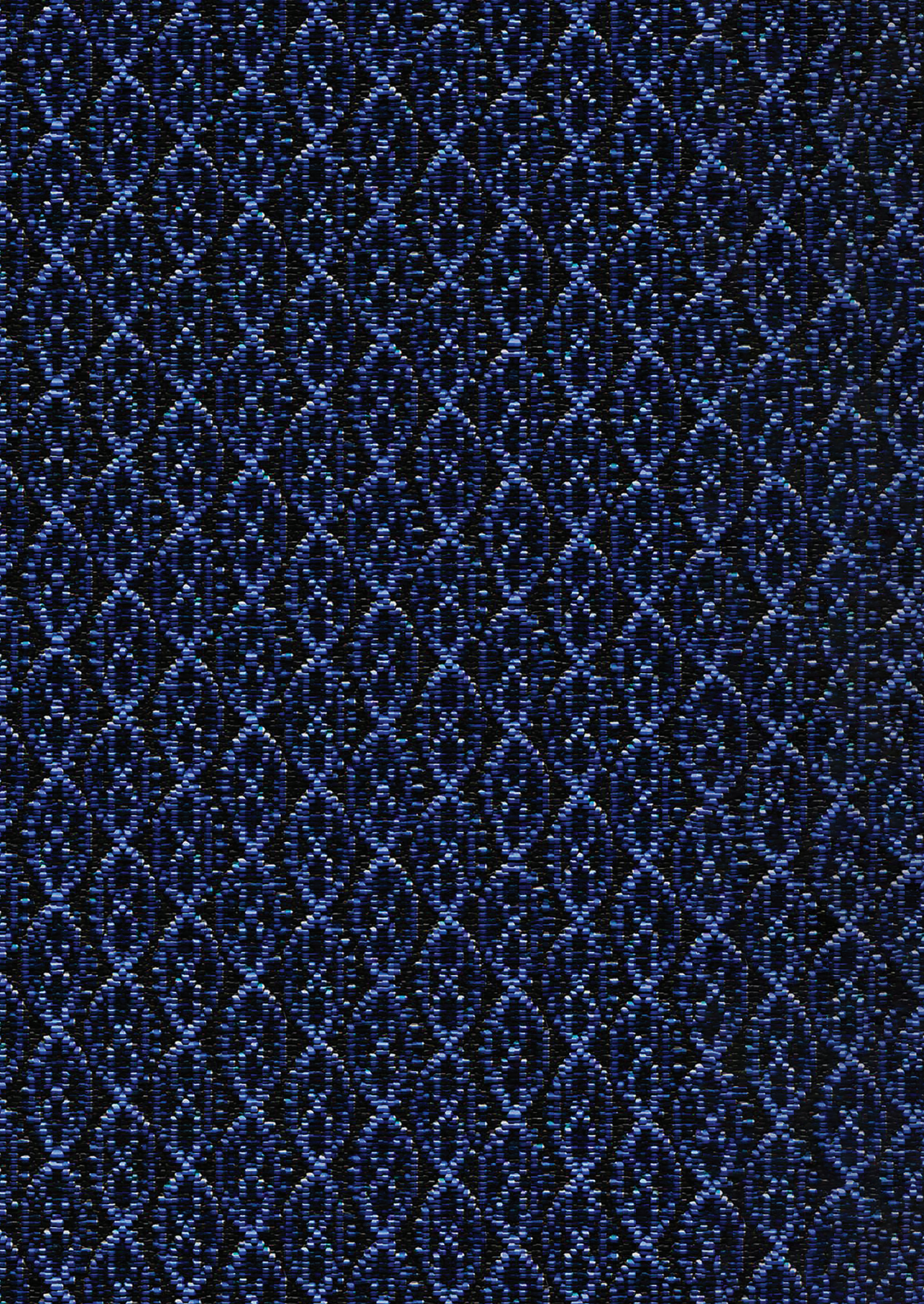

Part 7

Summary

Dementia with Lewy bodies (DLB) is a common neurodegenerative disease in the elderly. The disease is characterized by progressive cognitive decline accompanied by parkinsonism, hallucinations, fluctuating cognition, and REM-sleep behavior disorders. To date, no disease-modifying treatment options are available and the median survival of patients with DLB is approximately four years from diagnosis. The pathological hallmarks of DLB are cortical Lewy bodies and Lewy neurites. However, Alzheimer's disease (AD) pathology is also observed in the majority of patients. In addition to these pathological features, clinical symptoms and genetic factors also overlap with those of AD and Parkinson's disease (PD).

It is likely that genetic factors play a considerable role in DLB. Recently, the heritable component has been estimated to be approximately 60%. However, to date, only few genetic studies have been performed in DLB. The aim of this thesis was to shed more light on the genetics of DLB by studying 1) familial aggregation in DLB, 2) known AD and PD genes associated with DLB, and 3) novel genes associated with DLB.

Part 1 provides a general introduction to the thesis and summarizes the genetic research on DLB up until the beginning of this work in 2016. Up until that time, genetic studies were only performed in small study groups with candidate gene-based (biased) approaches. Unbiased genome wide association studies and whole exome sequencing (WES) studies had not yet been performed by then. The candidate gene studies showed that the *APOE* ε4 allele and specific variants in *GBA* and *SNCA* are associated with DLB. These genetic factors had previously been shown to play a role in AD (*APOE* ε4 allele) and PD (*GBA* and *SNCA*). Besides the fact that little was known about which genetic factors play a role in DLB, it was also largely unknown what effect these genetic factors have on the phenotype of DLB patients.

In **Part 2** we, therefore, examined the differences in phenotype between DLB patients with relatives with dementia or PD (familial patients) and DLB patients without relatives with these diseases (sporadic patients). We showed that familial DLB patients have a shorter survival than sporadic DLB patients. Furthermore, we demonstrated that a higher percentage of familial DLB patients have elevated AD biomarkers in their cerebrospinal fluid (CSF) compared to sporadic DLB patients. These findings suggest that genetic factors contribute to disease course, possibly by influencing the amount of concomitant AD pathology.

Owing to the genetic overlap between DLB, PD and AD, we investigated more profoundly if known genes associated with PD and AD also play a role in DLB. **Part 3** describes these genetic analyses in carefully selected study groups, namely familial DLB patients (**Chapter 3.1**) and pathologically confirmed DLB patients with a rapidly progressive disease course (**Chapter 3.2**). We showed that the *APOE* ε4 allele and specific variants in *GBA* are also important genetic risk factors in these patient groups.

The search for novel genes associated with DLB is described in **Part 4** and **Part 5**.

In **Part 4** we studied the association between the *LRP10* gene and DLB, and other related disorders. The *LRP10* gene was recently nominated as a novel gene associated with PD, PD dementia, and DLB. The function of LRP10 is largely elusive. However, the protein has been suggested to play a role in the aggregation of amyloid-β and α-synuclein. **Chapter 4.1** illustrates that rare, possibly pathogenic variants in *LRP10* are detected in clinically diagnosed PD and PD dementia patients from the South West of the Netherlands. In **Chapter 4.2** and **Chapter 4.3** we described that rare, possibly pathogenic *LRP10* variants may also play a role in patients with dementia and Lewy pathology, dementia patients with parkinsonism without Lewy pathology, and patients with progressive supranuclear palsy. In **Part 5**, we searched for novel genes by combining WES and CSF proteomics. **Chapter 5.1** outlines the identification of six novel CSF candidate biomarkers for DLB: VGF, SCG2, NPTX2, NPTXR, PDYN, and PCSK1N. These proteins play a role in, amongst others, synaptic dysfunction, and show promise to improve the diagnostic accuracy of DLB. The data from **Chapter 5.1** and the WES data were combined in the pilot study described in the **Appendix to Chapter 5.1.** The hypothesis behind this approach was that a pathogenic genetic variant will lead to a differential expression of the corresponding protein in the CSF. Although we identified overlap between eight genes and proteins, the evidence that these genes or proteins are associated with DLB remains very limited at this stage.

Part 6 provides a general discussion of the findings of this thesis in the context of the current literature and provides suggestions for future study directions.

Samenvatting

Dementie met Lewy bodies (DLB) is een vaak voorkomende neurodegeneratieve ziekte bij ouderen. De ziekte wordt gekenmerkt door progressieve cognitieve achteruitgang met hierbij parkinsonisme, een fluctuerend bewustzijn en REM-slaap gedragstoornissen. Er is geen medicatie die aangrijpt op het ziekteproces en de mediane overleving van patiënten met DLB is ongeveer 4 jaar vanaf de diagnose. De pathologische kenmerken van DLB zijn corticale Lewy bodies en Lewy neurieten. De meeste patiënten hebben daarnaast ook Alzheimer pathologie. Niet alleen pathologische kenmerken, maar ook klinische en genetische kenmerken overlappen met kenmerken die voorkomen bij de ziekte van Alzheimer (ZvA) en de ziekte van Parkinson (ZvP).

Genetische factoren lijken een belangrijke rol bij DLB te spelen. Recent is de erfelijke component zelfs geschat op ongeveer 60%. Er is echter nog maar weinig genetisch onderzoek bij DLB verricht. Het doel van dit proefschrift was om meer inzicht te krijgen in de genetica van DLB door het bestuderen van 1) het familiair voorkomen van DLB, 2) bekende ZvA en ZvP genen die geassocieerd zijn met DLB, en 3) nieuwe genen die geassocieerd zijn met DLB.

Deel 1 bevat een algemene introductie van het proefschrift en vat het genetisch onderzoek bij DLB samen tot de start van dit promotieonderzoek in 2016. Tot die tijd waren er alleen onderzoeken uitgevoerd in kleine studiegroepen waarbij gezocht werd naar varianten in kandidaatgenen. Genome wide association studies en whole exome sequencing (WES) studies waren tot dan toe nog niet uitgevoerd. De onderzoeken naar varianten in kandidaatgenen hebben aangetoond dat het *APOE* ε4 allel en specifieke varianten in *GBA* en *SNCA* geassocieerd zijn met DLB. Deze variaties zijn tevens genetische risicofactoren voor de ZvA *(APOE* ε4 allel) en de ZvP (*GBA* en *SNCA*). Naast dat er nog maar weinig bekend was over welke genetische factoren een rol spelen bij DLB, was het ook grotendeels onbekend welk effect deze genetische factoren hebben op de klinische kenmerken (fenotype) van patiënten met DLB.

In **Deel 2** hebben wij daarom de verschillen in fenotype tussen DLB patiënten met familieleden met dementie of de ZvP (familiaire patiënten) en DLB patiënten zonder familieleden met deze ziektes (sporadische patiënten) onderzocht. We hebben laten zien dat familiaire patiënten een kortere overleving hebben dan sporadische patiënten. Hiernaast hebben we laten zien dat relatief meer familiaire patiënten dan sporadische patiënten Alzheimer eiwitten in het hersenvocht hebben. Deze resultaten suggereren dat genetische factoren invloed hebben op het ziektebeloop, mogelijk door het beïnvloeden van Alzheimer pathologie.

Aangezien er een genetische overlap is tussen DLB, de ZvA en de ZvP, hebben wij verder onderzocht welke bekende genen die geassocieerd zijn met de ZvA en de ZvP ook een rol spelen bij DLB. **Deel 3** beschrijft deze genetische analyses bij zorgvuldig geselecteerde

Curriculum vitae

Leonie Vergouw was born on the 6th of November 1985 in Nieuwegein, the Netherlands. She went to the Gymnasium at the Oosterlicht College in Nieuwegein (secondary school). After secondary school, she obtained a Bachelor's degree in Chemical Engineering and Bioprocess Technology at the Technical University of Delft (2007), and a Bachelor's degree in Psychology at the University of Leiden and the University of Utrecht (2010). She also studied Medicine at the University of Utrecht and the Erasmus Medical Center in Rotterdam (2008-2014). She traveled a lot and did internships in Australia and Kenia. After obtaining her Master's degree in Medicine, she worked as medical doctor in Neurology (2014-2015) at the Sint Franciscus Gasthuis in Rotterdam. She started her PhD on the genetics of dementia with Lewy bodies in 2015, under supervision of Dr. F.J. de Jong, Prof. dr. J.C. van Swieten and Prof. dr. V. Bonifati. Leonie currently lives in Stellendam with her husband Michael and daughter Liva and started as medical doctor at the Clinical Genetics department in the Erasmus Medical Center in February 2020.

PhD portfolio

1. PhD training	Year	ECTS
General Courses		
Biostatistical Methods I: Basic Principles (NIHES)	2015	5.7
E-BROK	2015 & 2019	1.7
Biomedical English Writing (MolMed)	2016	2
Biomedical Research Techniques (MolMed)	2016	1.5
'R' statistical package (MolMed)	2016	1.8
Scientific Integrity	2017	0.3
Biomedical English Writing and Communication	2017	3
Masterclass English	2019	2
Presentation Skills Workshops	2019	1.5
Specific courses		
An introduction to the analysis of next-generation sequencing data (NIHES)	2015	1.4
Principles of genetic epidemiology (NIHES)	2015	0.7
Genomics in molecular medicine (NIHES)	2015	1.4
NeuroPathology course The 13th International Conference on Alzheimer's & Parkinson's Diseases	2017	0.3
Course European Confederation of Neuropathological Societies Neurodegeneration	2017	1.5
Introduction to Cytoscape	2018	0.5
Conferences and seminars		
International Parkinson and Movement Disorder Society Conference. Alpha-Synuclein: The Gateway to Parkinsonism	2015	1
International Dementia with Lewy Bodies Conference (poster presentation)	2015	1
Patiënt- en matelzorgersdag Lewy body dementie (organisation and oral presentation)	2015-2018	1
20th International Congress of Parkinson's Disease and Movement Disorders	2016	1.5
Dementie Update	2016	0.3
Mix and Match meeting Alzheimer Nederland (1 year: organisation)	2015-2017	0.5
The 13th International Conference on Alzheimer's & Parkinson's Diseases (poster presentation)	2017	1.5
Symposium: (Over)leven met dementie met Lewy bodies (oral presentation)	2017 & 2019	1
Publieksmiddag dementie (oral presentation)	2019	0.5
Other		
Research meeting, weekly	2015-2019	4
Sub Investigator medication trials	2015-2019	20
Creation of website www.lewy.nl	2015-2017	2
2. Teaching		
Lectures		
Tutor classes Medicine students	2015	1.5
Presentation Sint Franciscus Gasthuis	2016	0.3
Supervising Master's theses		
Five students	2015-2019	7.5
Total		**68.9**

List of publications

Vergouw LJM, Quadri M, van Steenoven I, Geut H, Breedveld GJ, Netherlands Brain Bank, van de Berg WDJ, Rozemuller AJM, Lemstra AW, van Swieten JC, Bonifati V, de Jong FJ. Familial Dementia with Lewy bodies: a comprehensive analysis of genes involved in Parkinson's or Alzheimer's Disease. *Submitted*

Vergouw LJM*, Geut H*, Breedveld G, Kuipers DJS, Quadri M, Rozemuller AJM, van Swieten JC, de Jong FJ, van de Berg WDJ, Bonifati V. Clinical and pathological phenotypes of patients with *LRP10* variants. *Under review*

van Steenoven I*, Koel-Simmelink MJA*, **Vergouw LJM**, Piersma SR, Pham TV, Ferri G-L, Cocco C, Noli B, Worley PF, Xiao M-F, Xu D, Oeckl P, Otto M, van der Flier WM, de Jong FJ, Jimenez CR, Lemstra AW, Teunissen CE. Identification of novel cerebrospinal fluid biomarker candidates for dementia with Lewy bodies: a proteomic approach. *Under review*

Vergouw LJM, Melhem S, Kaat LD, Chiu WZ, Breedveld G, Boon AJW, Quadri M, van Swieten JC, Bonifati V, de Jong FJ. *LRP10* variants in progressive supranuclear palsy. *Accepted, Neurobiology of Aging*

Vergouw LJM, Bosman B, Salomé M, Steenoven I, van de Beek M, Hoogers S, Roks G, Lemstra AW, van Swieten JC, de Jong FJ. Family history is associated with phenotype in dementia with Lewy bodies. *Journal of Alzheimer's Disease* 2020;73:269-275.

Vergouw LJM, Ruitenberg A, Wong TH, Melhem S, Breedveld GJ, de Jong FJ, Bonifati V, van Swieten JC, Quadri M. *LRP10* variants in Parkinson's disease and dementia with Lewy bodies in the South-West of the Netherlands. *Parkinsonism and Related Disorders* 2019;65:243-247.

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Quadri M, Mandemakers W, Grochowska MM, Masius R, Geut H, Fabrizio E, Breedveld GJ, Kuipers D, Minneboo M, **Vergouw LJM**, Carreras Mascaro A, Yonova-Doing E, Simons E, Zhao T, Di Fonzo AB, Chang HC, Parchi P, Melis M, Correia Guedes L, Criscuolo C, Thomas A, Brouwer RWW, Heijsman D, Ingrassia AMT, Calandra Buonaura G, Rood JP, Capellari S, Rozemuller AJ, Sarchioto M, Fen Chien H, Vanacore N, Olgiati S, Wu-Chou YH, Yeh TH, Boon AJW, Hoogers SE, Ghazvini M, IJpma AS, van IJcken WFJ, Onofrj M, Barone P, Nicholl DJ, Puschmann A, De Mari M, Kievit AJ, Barbosa E, De Michele G, Majoor-Krakauer D, van Swieten JC, de Jong FJ, Ferreira JJ, Cossu G, Lu CS, Meco G, Cortelli P, van de Berg WDJ, Bonifati V, in collaboration with the International Parkinsonism Genetics Network. LRP10 genetic variants in familial Parkinson's disease and dementia with Lewy bodies: a genome-wide linkage and sequencing study. *Lancet Neurology* 2018;17:597-608.

Vergouw LJM, Egal M, Bergmans B, Dippel DWJ, Lingsma HF, Vergouwen MDI, Willems PWA, Oldenbeuving AW, Bakker J, van der Jagt M. High early fluid input after aneurysmal subarachnoid hemorrhage: combined report of association with delayed cerebral ischemia and feasibility of cardiac output-guided fluid restriction. *Journal of Intensive Care Medicine* 2017;1:1-9.

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Lemstra AW, **Vergouw LJM**, Hoogers SE, de Jong FJ. Dementie met Lewy bodies (DLB). *Nervus, praktijkgerichte nascholing over neurologie* 2016;1:20-26.

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List of abbreviations

AAD	age at death
AAO	age at onset
AAS	age at sampling
AAV	adeno-associated viral vector
ABCA7	*ATP binding cassette subfamily A member 7* gene
AD	Alzheimer's disease / autosomal dominant
AHNAK	*AHNAK nucleoprotein* gene
ALS	amyotrophic lateral sclerosis
APOE	*apolipoprotein E* gene
APP	*amyloid precursor protein* gene
AR	autosomal reccessive
ARTAG	age-related astrogliopathy
$A\beta_{1\text{-}42}$	amyloid-$\beta_{1\text{-}42}$
B	benign
BNE	Brain Net Europe
BuChE	*butyrylcholinesterase* gene
C9orf72	*chromosome 9 open reading frame 72* gene
CA2	cornu ammonis region 2
CAA	cerebral amyloid angiopathy
CADD	Combined Annotation Dependent Depletion
CERAD	Consortium to Establish a Registry for Alzheimer's disease
CHCHD2	*coiled-coil-helix-coiled-coil-helix domain containing 2* gene
CHMP2B	*charged multivesicular body protein 2B* gene
CI	confidence interval
cingulate	posterior cingulate gyrus
CJD	Creutzfeldt-Jakob's disease
CLTC	*clathrin heavy chain* gene
CNV	copy number variation
CSF	cerebrospinal fluid
CV	coefficient of variation
D	damaging / disease causing
DaT-SPECT	^{123}I[FP-CIT] single photon emission computed tomography
DLB	dementia with Lewy bodies
e.g.	exempli gratia (for example)
EEG	electro-encephalogram

EIF4G1	*eukaryotic translation initiation factor 4 gamma 1* gene
ELISA	enzyme-linked immunosorbent assay
ExAC-NFE	Exome Aggregation Consortium - Non Finnish Europeans
EXT2	*exostosin glycosyltransferase 2* gene
FATHMM	Functional Analysis Through Hidden Markov Models
FCGBP	*Fc fragment of IgG binding protein* gene
FDR	false discovery rate
FREM2	*FRAS1 related extracellular matrix protein 2* gene
frontal	frontal gyrus (Brodmann area 10)
FTD	frontotemporal dementia
GATK	Genome Analysis Toolkit
GBA	*glucocerebrosidase* gene
Gcase	glucocerebrosidase
GERP	Genomic Evolutionary Rate Profiling
GGA	golgi associated, gamma adaptin ear containing, ARF binding protein
GIGYF2	*GRB10 interacting GYF protein 2* gene
GlcCer	glucosylceramide
GLSph	glucosylsphingosine
GnomAD	Genome Aggregation Database
GoNL	Genome of the Netherlands
GRCh37	genome reference consortium human 37
GRN	*granulin* gene
GWA	genome wide association
HEG1	*heart development protein with EGF like domains 1* gene
HEX	Healthy Exomes
i.e.	id est (in other words)
IQR	interquartile range
KRT73	*keratin 73* gene
L	low
LB	Lewy body / likely benign
LBD	Lewy body disease
LC	locus coeruleus / liquid chromatography
LFQ	label-free quantification
LN	Lewy neurite
LR	logistic regression
LRP10	*low-density lipoprotein receptor related protein 10* gene
LRRK2	*leucine-rich repeat kinase 2* gene

LRT	Likelihood Ratio Test
M	medium
MAF	minor allele frequency
MAPT	*microtubule-associated protein tau* gene
M-CAP	Mendelian Clinically Applicable Pathogenicity
MCI	mild cognitive impairment
MEC	medical ethics committtee
MLPA	Multiplex Ligation dependent Probe Amplification
MMSE	Mini-Mental State Examination
MR-DWI	magnetic resonance diffusion weighted imaging
MRI	magnetic resonance imaging
MS	multiple sclerosis
MSA	multiple system atrophy
MS/MS	tandem mass spectrometry
MTA	medial temporal lobe atrophy
N	polymorphism / neutral
n.a.	not applicable
NA	not available
ND	not done
NIA-AA	National Instittue on Aging - Alzheimer Association
NPTX2	neuronal pentraxin 2
NPTXR	neuronal pentraxin receptor
P	polymorphism automatic
parietal	inferior parietal lobe
PARK2	*parkin* gene
PCSK1N	ProSAAS
PC	prohormone convertase
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PDYN	proenkephalin-B
PINK1	*PTEN induced putative kinase 1* gene
PolyPhen2 HDIV	Polymorphism Phenotyping version 2 human diversity
PolyPhen2 HVA	Polymorphism Phenotyping version 2 human variation
PP	probably pathogenic
PRKAR1B	*protein kinase cAMP-dependent type I regulatory subunit beta* gene
PRNP	*prion protein* gene
PSEN1	*presenilin 1* gene
PSEN2	*presenilin 2* gene

PSP	progressive supranuclear palsy
PSWC	periodic sharp wave complex
p-tau	tau phosphorylated at threonine 181
RBD	rapid eye movement-sleep behaviour disorder
Ref	reference
REM	rapid eye movement
SCARB2	*scavenger receptor class B member 2* gene
SCG2	secretogranin-2
SD	standard deviation
SE	standard error
SIFT	Sorting Intolerant From Tolerant
SN	substantia nigra
SNCA	*α-synuclein* gene
SNCB	*β-synuclein* gene
SNP	single nucleotide polymorphism
SORL1	*sortilin related receptor 1* gene
SQSTM1	*sequestosome* gene
SRM	selected reaction monitoring
STR	short tandem repeat
SVM	Support Vector Machine
SYNE1	*spectrin repeat containing nuclear envelope protein 1* gene
T	tolerated
temp-occ	temporo-occipital cortex
temporal	medial temporal gyrus
TREM2	*triggering receptor expressed on myeloid cells 2* gene
t-tau	total tau
UN	unknown
var / V	variant
VGF	neurosecretory protein VGF
VPS13C	*vacuolar protein sorting 13 homolog C* gene
VPS35	*vacuolar protein sorting-associated protein 35* gene
WES	whole exome sequencing
WGS	whole genome sequencing
WHO	World Health Organization
wt	wild-type