Published in final edited form as:

Neurobiol Dis. 2016 October ; 94: 55–62. doi:10.1016/j.nbd.2016.06.004.

Next-generation sequencing reveals substantial genetic contribution to dementia with Lewy bodies

Joshua T. Geiger^a, Jinhui Ding^b, Barbara Crain^c, Olga Pletnikova^c, Christopher Letson^b, Ted M. Dawson^{d,e,f,g}, Liana S. Rosenthal^d, Alexander Pantelyat^d, J. Raphael Gibbs^b, Marilyn S. Albert^d, Dena G. Hernandez^b, Argye E. Hillis^d, David J. Stone^h, Andrew B. Singleton^b, North American Brain Expression Consortium, John A. Hardyⁱ, Juan C. Troncoso^{c,d}, and Sonja W. Scholz^{a,b,d,*}

^aNeurodegenerative Diseases Research Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA

^bLaboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA

^cDepartment of Pathology (Neuropathology), Johns Hopkins University School of Medicine, Baltimore, MD, USA

^dDepartment of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

^eSolomon H. Synder Department of Neuroscience, Johns Hopkins University, Baltimore, MD, USA

^fDepartment of Pharmacology and Molecular Science, Johns Hopkins University School of Medicine, Baltimore, MD, USA

^gNeuroregeneration Program, Institute of Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA

^hGenetics and Pharmacogenomics, Merck Research Laboratories, West Point, PA, USA

ⁱDepartment of Molecular Neuroscience, University College London, London, UK

Abstract

Dementia with Lewy bodies (DLB) is the second most common neurodegenerative dementia after Alzheimer's disease. Although an increasing number of genetic factors have been connected to this debilitating condition, the proportion of cases that can be attributed to distinct genetic defects

*Corresponding author: Sonja W. Scholz, MD, PhD; Neurodegenerative Diseases Research Unit, NINDS, National Institutes of Health; 35 Convent Drive, Bethesda, MD 20892-3707, USA; Tel: +1 (301)-435-1788; Fax: +1 (301)-451-7295; sonja.scholz@nih.gov.

Disclosure statement

Argye Hillis serves as a paid member of the Data Safety Monitoring Board of clinical trials by Axovant Sciences Ltd. Dr. Hillis receives fees from the American Heart Association as Associate Editor of Stroke and from Elsevier as Associate Editor of Practice Update. Liana Rosenthal received salary support in the previous 12 months from the JHU Morris K. Udall Parkinson's Disease Research Center of Excellence NIH/NINDS P50 NS038377, the Johns Hopkins Biomarker Initiative (NIH/NINDS U01 NS082133), the Marilyn and Edward Macklin Foundation, and the Michael J. Fox Foundation. She also received an honorarium from the Edmond J. Safra Foundation and Functional Neuromodulation. None of the other authors report any conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

is unknown. To provide a comprehensive analysis of the frequency and spectrum of pathogenic missense mutations and coding risk variants in nine genes previously implicated in DLB, we performed exome sequencing in 111 pathologically confirmed DLB patients. All patients were Caucasian individuals from North America. Allele frequencies of identified missense mutations were compared to 222 control exomes. Remarkably, ~25% of cases were found to carry a pathogenic mutation or risk variant in *APP*, *GBA* or *PSEN1*, highlighting that genetic defects play a central role in the pathogenesis of this common neurodegenerative disorder. In total, 13% of our cohort carried a pathogenic mutation in *GBA*, 10% of cases carried a risk variant or mutation in *PSEN1*, and 2% were found to carry an *APP* mutation. The *APOE ε4* risk allele was significantly overrepresented in DLB patients (p -value <0.001). Our results conclusively show that mutations in *GBA*, *PSEN1*, and *APP* are common in DLB and consideration should be given to offer genetic testing to patients diagnosed with Lewy body dementia.

Graphical abstract

Frequency of pathogenic mutations and risk variants in *GBA*, *PSEN1* and *APP* in DLB

1. Introduction

Dementia with Lewy bodies (DLB) is the second most common neurodegenerative dementia after Alzheimer's disease (Lippa, et al., 2007), clinically characterized by a combination of progressive cognitive decline, fluctuating mental status, parkinsonism and visual hallucinations. Pathologically, brains of DLB patients demonstrate widespread Lewy body pathology, and the vast majority of patients have coexisting neurofibrillary tangles and amyloid plaques sufficient to meet the neuropathological criteria for Alzheimer dementia (McKeith, et al., 2005). These pathological findings place DLB midway along a spectrum between Parkinson disease and Alzheimer dementia (Berg, et al., 2014).

Genetic data provide additional support for DLB existing along this Parkinson disease/Alzheimer dementia continuum. Mutations in five Parkinson disease genes have been linked to the DLB phenotype, including genetic variation in *GBA*, *LRRK2*, *MAPT*, *SCARB2* and *SNCA* (Bras, et al., 2014; Colom-Cadena, et al., 2013; Denson, et al., 1997; Fuchs, et al., 2007; Gwinn-Hardy, et al., 2000; Ishikawa, et al., 1997; Nalls, et al., 2013; Ohara, et al., 1999; Singleton, et al., 2003; Zarranz, et al., 2004; Zimprich, et al., 2004). Advances in

Alzheimer dementia genetics have provided additional insights into the molecular pathogenesis of DLB. For instance, the *APOE* $\epsilon 4$ allele is a significant risk factor for DLB (Tsuang, et al., 2013), and familial Alzheimer dementia cases due to *APP*, *PSEN1* and *PSEN2* mutations occasionally present with mixed Alzheimer and Lewy body pathology raising the possibility of a shared molecular predisposition between Alzheimer dementia and DLB (Ishikawa, et al., 2005; Leverenz, et al., 2006; Meeus, et al., 2012).

Despite these insights into the genetics of DLB, the frequency at which these mutations occur in patients diagnosed with DLB is poorly understood. To fill this gap in our knowledge, we explored the frequencies and spectrum of mutations in genes previously implicated in DLB (*GBA*, *LRRK2*, *MAPT*, *APOE*, *APP*, *PSEN1*, *PSEN2*, *SCARB2*, *SNCA*) using exome sequence data generated for a cohort of patients with pathologically confirmed DLB.

2. Material and methods

2.1 Subjects

A total of 111 cases with extensive Lewy body pathology were obtained from the Johns Hopkins Morris K. Udall Center of Excellence for Parkinson's Disease Research and the Johns Hopkins Alzheimer Disease Research Center. These samples were characterized by widespread Lewy body pathology and met criteria for either neocortical (n = 86 cases) or transitional-type DLB (n=25 cases) using the McKeith classification (McKeith, et al., 2005). The majority of patients (69%) also met pathological criteria for Alzheimer dementia (Geiger, et al., 2016). All subjects were Caucasian, with males constituting 75% of the cohort. The average age at symptom onset was 65 (sd $\pm$ 10) years and mean age at death was 78 ($\pm$ 8) years. Thirty-three patients (30% of the entire cohort) had a family history of cognitive impairment or parkinsonism in at least one first- or second-degree relative.

We used in-house control exomes of 222 neurologically normal individuals from the North American Brain Expression Consortium. Sample acquisition for this cohort has been described elsewhere (Hernandez, et al., 2012). All control subjects were Caucasian, with males constituting 66% of the cohort.

The institutional review board approved the study, and written informed consent was obtained for each patient.

2.2 Sample preparation, exome capture and sequencing

DNA was extracted from frozen brain tissue of each subject using the DNeasy extraction kit (Quiagen, Valencia, CA). Exome capture was performed on each subject using Nextera enrichment technology (Expanded Exome Oligo kit v4; Illumina, San Diego, CA). This exome capture kit targets the expanded exome, consisting of the 2% of the human genome coding for exons, UTRs and miRNAs. Exome libraries were indexed, and a total of 12 libraries were pooled for high-throughput, 125 bp paired-end sequencing (TruSeq v4 kit) on an Illumina HiSeq 2500 platform. Raw sequencing data were uploaded into BaseSpace (Illumina Inc., CA), a genomic cloud-computing interface. Sequence data of pooled libraries were de-multiplexed using CASAVA v1.8.2 (Illumina), followed by alignment to the human

reference genome (build hg19) using the Burroughs-Wheeler aligner (Li and Durbin, 2009). Next, genotypes were called from aligned sequences following Genome Analysis Toolkit (version 3) best practices (McKenna, et al., 2010). Quality control steps were performed in PLINK 1.90 (Purcell, et al., 2007). These included estimation of coverage, call rate, heterozygosity (to rule out contamination), population (to confirm Caucasian ancestry), cryptic relatedness and phenotype-genotype gender matching. None of the samples were excluded based on these stringent quality control metrics.

2.3 Filtering and annotating missense mutations

All exomes were of high quality with a 10x coverage > 90% and a 30x coverage > 80% (details about the coverage for each of the nine genes studied is shown in Supplementary Figure 1 and Supplementary Table 1). VCFtools (version 0.1.13) (Danecek, et al., 2011) was used to extract missense mutations in the following genes: *APOE*, *APP*, *GBA*, *LRRK2*, *MAPT*, *PSEN1*, *PSEN2*, *SCARB2* and *SNCA*. All variants were annotated in SeattleSeq (snp.gs.washington.edu/SeattleSeqAnnotation138/) and ANNOVAR (version 2015-06-17) (Wang, et al., 2010). We evaluated the frequencies of identified missense mutations in the European ExAC population (version 0.3; exac.broadinstitute.org) and in 222 in-house neurologically normal controls. Protein change predictions were determined using SIFT, PolyPhen-2, and MutationTaster2 (Adzhubei, et al., 2010; Ng and Henikoff, 2003; Schwarz, et al., 2014). Mutations were described according to human genome variation society nomenclature guidelines (www.hgvs.org/mutnomen) (den Dunnen and Antonarakis, 2000). *GBA* variants are listed with the traditional amino-acid residue numbering in square brackets (excluding the signal peptide).

2.4 Confirmatory Sanger sequencing and Taqman genotyping

Identified missense mutations in the DLB cohort were sequenced using the Big-Dye Terminator v3.1 sequencing kit (Applied Biosystems Inc., Foster City, CA, USA), run on an ABI 3730xl genetic analyzer, and analyzed using Sequencher software (version 5.1, Gene Codes Corporation, Ann Arbor, MI, USA). PCR primers and conditions are listed in Supplementary Table 2. *APOE* rs7412 (p.R176C) and rs429358 (p.C130R) were genotyped using an established TaqMan method (Applied Biosystems Inc., Foster City, CA, USA) (Federoff, et al., 2012).

2.5 Pathogenicity determination

Pathogenicity of coding variants was determined based on: 1) literature review implicating a given variant with neurodegenerative disease (Alzheimer dementia, parkinsonism, Gaucher disease, Lewy body dementia or other types of dementia) and 2) in-silico modeling (predicting pathogenicity in at least one of three prediction tools: SIFT, PolyPhen2, Mutation Taster). In addition, an increased minor allele frequency in cases compared to controls was interpreted as supportive for disease-association.

2.6 Mutation mapping and in-silico protein modeling

Mutations were mapped to the reference sequences using FancyGene (Rambaldi and Ciccarelli, 2009) and illustrated in Adobe Illustrator CC (version 19.1.0). Protein modeling

was performed in PyMOL software (v1.7.6, Schrödinger LLC; www.pymol.org) using previously described protein structures in the Protein Data Bank (Bai, et al., 2015; Barrett, et al., 2012; Berman, et al., 2000; Chen, et al., 2011; Dvir, et al., 2003; Huxford, et al., 1998).

3. Results

We performed exome sequencing in a cohort of 111 pathologically confirmed DLB patients, and examined the mutation rate in nine genes that had been previously linked to this type of neurodegeneration. In total, we identified eleven missense mutations in the genes *GBA*, *PSEN1* and *APP* in nearly 25% of the cohort that are either disease-causing or high-risk variants (Table 1, Figure 1). In addition, we confirmed a significant overrepresentation of the *APOE ε4* risk allele in DLB. To rule out false positive findings, we confirmed all identified variants by direct Sanger sequencing or Taqman genotyping.

3.1 Disease-associated mutations by individual genes

3.1.1 GBA—We identified fourteen patients (Table 2) with one of the following pathogenic *GBA* mutations: p.D448H [p.D409H], p.N409S [p.N370S], p.E365K [p.E326K], p.R296Q [p.R257Q], and p.R87Q [p.R48Q]. The p.D448H [p.D409H], p.N409S [p.N370S] and p.R296Q [p.R257Q] *GBA* mutations have been associated with Gaucher disease and parkinsonism, likely due to impaired lysosomal protein degradation (Beutler, et al., 1994; Choi, et al., 2012; Sidransky, 2004; Sidransky, et al., 2009). The *GBA* p.E365K [p.E326K] mutation is considered a mild mutation as it has been demonstrated to reduce rather than abolish glucocerebrosidase enzyme activity (Alcalay, et al., 2015; Malini, et al., 2014). As such, homozygosity for this mutation is not sufficient to cause Gaucher disease; however, an increased frequency of heterozygous carriers has been demonstrated in cohorts of Parkinson disease supporting the notion that this mutation is pathogenic (Duran, et al., 2013; Nichols, et al., 2009). The *GBA* mutation p.R87Q [p.R48Q] has been previously described in a patient with Gaucher disease, but a role in parkinsonism for this particular rare mutation has not yet been reported (Rozenberg, et al., 2006). In total, fourteen DLB cases or 13% of the entire cohort were heterozygous for a pathogenic *GBA* mutation. No homozygous *GBA* mutation carriers or compound heterozygous patients were identified.

3.1.2 PSEN1—In *PSEN1*, we found missense mutations in eleven patients. The p.G206A mutation, which was present in one patient (patient 20; Table 2), is a known cause of familial Alzheimer dementia (AD) (Rogaeva, et al., 2001). The second mutation (p.E318G), which was present in ten patients, has been associated with significantly increased risk for AD in *APOE ε4* carriers (Benitez, et al., 2013). This variant was significantly overrepresented in the DLB cohort compared to control exomes (p-value 0.035, Fisher's exact test, OR 2.1, CI 1.035 – 3.758; supplementary table 4), suggesting that it likely constitutes a risk variant. In total, 10% of our DLB cohort carried a mutation in *PSEN1*. Interestingly, one patient (patient 14; Table 2) carried both a p.E318G *PSEN1* variant and a p.N409S *GBA* mutation. This individual presented with parkinsonism at age 40 and later developed cognitive impairment meeting criteria for Parkinson disease dementia. He had no family history of dementia or parkinsonism, and his pathology demonstrated pure DLB without any co-existing Alzheimer pathology.

3.1.3 APP—We detected two patients with disease-associated mutations in *APP*. One patient carried the highly penetrant p.V717I mutation, a known cause of familial AD with co-existing Lewy body pathology that has been shown to alter APP protein processing and tau expression (Halliday, et al., 1997; Lantos, et al., 1994; Muratore, et al., 2014). This mutation is located in the transmembrane domain of APP in close proximity to the γ -secretase cleavage site. The patient had a family history of early-onset dementia and pathology examination of her brain revealed extensive Lewy body pathology (neocortical-type DLB) as well as severe Alzheimer pathology (Braak stage 6, CERAD score C) (table 2). The second *APP* mutation we detected, p.E599K, has been previously associated with parkinsonism (Schulte, et al., 2015) and likely constitutes a risk variant. In total, 2% of our cohort carried an *APP* missense mutation.

3.1.4 APOE—The following variants were detected in *APOE*: p.C130R, p.R176C and p.L46P. The p.C130R and p.R176C variants make up the *APOE* $\epsilon 4$ risk allele, a known high-risk allele for AD and DLB (Hardy, et al., 1994). *APOE* p.L46P is a rare variant that has been shown to be in complete linkage disequilibrium with the *APOE* $\epsilon 4$ risk allele, and studies have demonstrated that this variant has no additional effect on risk of developing AD independent of the $\epsilon 4$ allele (Baron, et al., 2003; Kamboh, et al., 1999). In line with previous studies, the *APOE* $\epsilon 4$ allele was significantly overrepresented in our DLB cohort (25 heterozygous carriers, 10 homozygous carriers) compared to neurologically normal in-house controls (Fisher exact test, p-value < 0.001). Survival estimates comparing *APOE* $\epsilon 4$ carriers with *APOE* non-carriers demonstrated significantly shortened survival in *APOE* $\epsilon 4$ carriers (Supplementary Figure 2).

3.1.5 MAPT, LRRK2, PSEN2, SCARB2, SNCA—Pathogenic coding variants were not identified in *MAPT*, *LRRK2*, *PSEN2*, *SCARB2* or *SNCA*. Coding polymorphisms detected in our DLB cohort are listed in Supplementary Table 3.

3.2 Clinicopathologic features of mutation carriers

In total, twenty-six patients with causative mutations or high-risk variants in *APP*, *GBA* or *PSEN1* were identified. Clinical and pathological characteristics of these patients are shown in Table 2. Fourteen patients were male, and twelve were female. The average age at onset was 62 years (range: 40 – 82 years) and the mean age at death was 76 years (range: 57 – 91 years).

4. Conclusions

A substantial proportion of patients (~25% of the entire cohort) were found to carry disease-associated coding variants in the genes *GBA*, *PSEN1*, or *APP*, with mutations in *GBA* and *PSEN1* being the most frequent molecular defects, accounting for 13% and 10% of the cohort, respectively. The frequency of *GBA* mutations identified in our DLB cohort is comparable to the frequency found in Parkinson disease (Sidransky and Lopez, 2012). There are ongoing debates as to whether heterozygous, pathogenic *GBA* mutations constitute high-risk variants or dominant causative mutations with decreased penetrance (Anheim, et al., 2012; Sidransky, et al., 2009). This study does not resolve this controversy; nonetheless the

high frequency of pathogenic *GBA* mutations emphasizes a prominent role of lysosomal dysfunction in the pathogenesis of DLB. Only one of the two identified pathogenic *PSEN1* mutations, p.G206A, has been previously reported to cause familial dementia (Rogaeva, et al., 2001), whereas the second mutation, p.E318G, likely constitutes a risk variant rather than a causative mutation.

Along the same lines, only one of the two pathogenic *APP* mutations is a clearly causative mutation (p.V717I), whereas the p.E599K mutation is likely a risk variant. In support of prior evidence (Keogh, et al., 2016; Tsuang, et al., 2013), the frequency of the *APOE ε4* risk allele was significantly higher in our DLB cohort compared to Caucasian controls (p -value < 0.001) and survival was significantly shorter in *APOE ε4* carriers (Supplementary Figure 2). Interestingly, we found no pathogenic mutations in *LRRK2*, *MAPT*, *PSEN2*, *SCARB2*, and *SNCA* indicating that mutations in these genes are not a frequent cause of DLB. Taken together, these findings emphasize that molecular genetic defects play a significant role in the pathogenesis of this devastating neurological disease, and firmly place DLB along a continuum between Parkinson disease and Alzheimer dementia.

A major strength of our study was the use of a cohort of pathologically defined DLB. The clinical diagnosis of DLB is known to be inaccurate, primarily due to the heterogeneous clinical presentation observed among these patients and the difficulty of distinguishing mimic syndromes. The situation is further complicated by the one-year clinical rule used to separate Lewy body dementia into DLB and Parkinson disease dementia. According to this controversial guideline, the clinical diagnosis of DLB is only given if dementia occurs prior to or within one year of onset of parkinsonism. If the dementia occurs after this time point, a clinical diagnosis of Parkinson disease dementia is applied. In striking contrast to these arbitrary clinical definitions, however, DLB and Parkinson disease dementia are pathologically indistinguishable. Our data, which are based on a pathologically defined Caucasian cohort, clearly show that knowledge of genetics will be helpful in establishing the clinical diagnosis in cases of Lewy body dementia and may resolve the need for the one-year rule.

The clinical presentation of mutation carriers in our pathological defined DLB cohort was variable (Table 2) and, indeed, patients received a variety of clinical diagnoses prior to death and neuropathological examination. Clinical misdiagnosis is not unusual for this disease group, and it recapitulates the ongoing challenge faced by clinicians in attempting to diagnose a behaviorally heterogeneous patient population. The diversity of clinical presentations associated with extensive Lewy body pathology likely reflects variable extent of neuronal degeneration, α -synuclein, tau and amyloid aggregation in individual patients (Kim, et al., 2014).

Interestingly, only eleven out of twenty-six cases with a disease-associated mutations (42%) reported a positive family history of cognitive impairment or parkinsonism in first- or second-degree relatives. This observation illustrates an emerging concept in neurodegenerative diseases of late adulthood: namely, the absence of a family history does not exclude a genetic cause/predisposition (Scholz and Bras, 2015; Shulman, et al., 2011). Possible explanations for lack of a family history include death of relatives prior to

manifesting symptoms, phenotypic heterogeneity, somatic mutations, spontaneous mutations, reduced penetrance, or non-paternity. Another possible mechanism for seemingly sporadic disease is the occurrence of multiple molecular hits in a given individual (Escott-Price, et al., 2015; Reitz, et al., 2011; van Blitterswijk, et al., 2012). This polygenic inheritance concept is supported by observations in this study; specifically, three sporadic DLB patients (table 2: subjects 2, 6, 16) carried mutations in *GBA* or *PSEN1* in addition to the *APOE ε4* risk allele. Another patient (table 2: subject 14) carried two mutations, one causative mutation in *GBA* and one risk-variant in *PSEN1*, indicating that multiple molecular events could predispose a given individual to developing disease. The combination of such molecular hits may indeed determine where along the clinical Parkinson disease – DLB – Alzheimer disease continuum a patient falls.

Another strength of this study is the use of exome-sequencing technologies to rapidly screen several genes simultaneously. Exome sequencing has already been shown to be a powerful tool for discovering Mendelian forms of disease (Johnson, et al., 2010; Sailer, et al., 2012), but increasingly applications for complex diseases, such as in this study, are recognized. This study was designed to identify frequent causative mutations and coding risk variants in genes previously implicated in DLB, which also explains some of the limitations. Rare variants could have been missed, and additional studies in larger cohorts will be necessary for a more refined resolution of the genetic risk profile in DLB. Likewise, this study was not powered to perform gene-burden testing on a genome-wide level to identify possible novel disease genes involved in the pathobiology of DLB. For this reason, we focused on genes that have already been implicated in DLB to dissect the frequency at which mutations in these genes occur.

After completion of our analysis, a candidate gene study of exome data from a British DLB cohort was published (Keogh, et al., 2016). Similar to our findings, this study found an increased frequency of the *APOE ε4* risk allele. In addition, 5.7% of their study cohort carried a pathogenic *GBA* mutation. This frequency is lower than the frequency observed in our study (13%), which is likely explained by population heterogeneity. Another interesting observation in the study by Keogh and colleagues was the finding that one patient carried a rare pathogenic mutation in *CHMP2B* that had been previously described in cases with frontotemporal dementia (Isaacs, et al., 2011). This finding suggests a mechanistic overlap between the neurodegenerative dementias. We therefore queried our exome data for missense mutations in *CHMP2B*, but found no pathogenic variants in our North American cohort. Additional possibly pathogenic variants were predicted based on in-silico modeling in the genes *SQSTM1*, *EIF4G1*, *GIGYF2* and *PARK2*, but the evidence linking these genes to DLB is hypothetical.

In summary, our results suggest that consideration should be given to offer genetic counseling and testing to patients diagnosed with Lewy body dementia, given the substantial proportion of pathogenic mutations and risk variant carriers identified in this pathologically proven cohort. As we are entering the precision medicine era, refining a diagnosis by testing for molecular genetic defects is rapidly emerging as established practice. Characterization of common genetic defects in these patients is not only valuable for diagnostic considerations,

but may be valuable for prediction of the disease course, disease modeling, rational therapeutic interventions and ultimately disease prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding/Support: This work was supported (in part) by the Intramural Research Program of the National Institutes of Health (National Institute of Neurological Disorders and Stroke, National Institute on Aging; project Z01 AG000949). This work was also supported by Merck Research Laboratories. S.W.S. received a R25 career development grant by the National Institute of Neurological Disorders and Stroke (grant number: R25 NS065729).

Group Information: Control exome data were provided by the North American Brain Expression Consortium, which included the following members: Sampath Arepalli, Mark R Cookson, Allissa Dillman, Luigi Ferrucci, J Raphael Gibbs, Dena G Hernandez, Robert Johnson, Dan L Longo, Michael A Nalls, Richard O'Brien, Andrew Singleton, Bryan Traynor, Juan Troncoso, Marcel van der Brug, H Ronald Zielke and Alan Zonderman.

Additional contributions: Tissue samples for exome sequencing were provided by the Johns Hopkins Morris K. Udall Center of Excellence for Parkinson's Disease Research (NIH P50 NS38377) and the Johns Hopkins Alzheimer Disease Research Center (NIH P50 AG05146). The authors thank Ms. Gay Rudow for performing DNA extractions. Dr. Dawson is the Leonard and Madlyn Abramson Professor in Neurodegenerative Disease.

References

- Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. *Nature methods*. 2010; 7(4): 248–9. DOI: 10.1038/nmeth0410-248 [PubMed: 20354512]
- Alcalay RN, Levy OA, Waters CC, Fahn S, Ford B, Kuo SH, Mazzoni P, Pauciulo MW, Nichols WC, Gan-Or Z, Rouleau GA, Chung WK, Wolf P, Oliva P, Keutzer J, Marder K, Zhang X. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. *Brain*. 2015; 138(Pt 9):2648–58. DOI: 10.1093/brain/awv179 [PubMed: 26117366]
- Anheim M, Elbaz A, Lesage S, Durr A, Condroyer C, Viallet F, Pollak P, Bonaiti B, Bonaiti-Pellie C, Brice A. French Parkinson Disease Genetic G. Penetrance of Parkinson disease in glucocerebrosidase gene mutation carriers. *Neurology*. 2012; 78(6):417–20. DOI: 10.1212/WNL.0b013e318245f476 [PubMed: 22282650]
- Bai XC, Yan C, Yang G, Lu P, Ma D, Sun L, Zhou R, Scheres SH, Shi Y. An atomic structure of human gamma-secretase. *Nature*. 2015; 525(7568):212–7. DOI: 10.1038/nature14892 [PubMed: 26280335]
- Baron M, Jimenez-Escrig A, Orensanz L, Simon J, Perez-Tur J. Apolipoprotein E Pittsburgh variant is not associated with the risk of late-onset Alzheimer's disease in a Spanish population. *Am J Med Genet B Neuropsychiatr Genet*. 2003; 120B(1):121–4. DOI: 10.1002/ajmg.b.20028 [PubMed: 12815750]
- Barrett PJ, Song Y, Van Horn WD, Hustedt EJ, Schafer JM, Hadziselimovic A, Beel AJ, Sanders CR. The amyloid precursor protein has a flexible transmembrane domain and binds cholesterol. *Science*. 2012; 336(6085):1168–71. DOI: 10.1126/science.1219988 [PubMed: 22654059]
- Benitez BA, Karch CM, Cai Y, Jin SC, Cooper B, Carrell D, Bertelsen S, Chibnik L, Schneider JA, Bennett DA, Fagan AM, Holtzman D, Morris JC, Goate AM, Cruchaga C. Alzheimer's Disease Neuroimaging I Genetic Environmental Risk for Alzheimer's Disease Consortium G. The PSEN1, p.E318G variant increases the risk of Alzheimer's disease in APOE-epsilon4 carriers. *PLoS genetics*. 2013; 9(8):e1003685.doi: 10.1371/journal.pgen.1003685 [PubMed: 23990795]
- Berg D, Postuma RB, Bloem B, Chan P, Dubois B, Gasser T, Goetz CG, Halliday GM, Hardy J, Lang AE, Litvan I, Marek K, Obeso J, Oertel W, Olanow CW, Poewe W, Stern M, Deuschl G. Time to redefine PD? Introductory statement of the MDS Task Force on the definition of Parkinson's disease. *Mov Disord*. 2014; 29(4):454–62. DOI: 10.1002/mds.25844 [PubMed: 24619848]

- Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. *Nucleic Acids Res.* 2000; 28(1):235–42. [PubMed: 10592235]
- Beutler E, Demina A, Gelbart T. Glucocerebrosidase mutations in Gaucher disease. *Mol Med.* 1994; 1(1):82–92. [PubMed: 8790604]
- Bras J, Guerreiro R, Darwent L, Parkkinen L, Ansorge O, Escott-Price V, Hernandez DG, Nalls MA, Clark LN, Honig LS, Marder K, Van Der Flier WM, Lemstra A, Scheltens P, Rogaeva E, St George-Hyslop P, Londos E, Zetterberg H, Ortega-Cubero S, Pastor P, Ferman TJ, Graff-Radford NR, Ross OA, Barber I, Braae A, Brown K, Morgan K, Maetzler W, Berg D, Troakes C, Al-Sarraj S, Lashley T, Compta Y, Revesz T, Lees A, Cairns N, Halliday GM, Mann D, Pickering-Brown S, Dickson DW, Singleton A, Hardy J. Genetic analysis implicates APOE, SNCA and suggests lysosomal dysfunction in the etiology of dementia with Lewy bodies. *Hum Mol Genet.* 2014; doi: 10.1093/hmg/ddu334
- Chen J, Li Q, Wang J. Topology of human apolipoprotein E3 uniquely regulates its diverse biological functions. *Proc Natl Acad Sci U S A.* 2011; 108(36):14813–8. DOI: 10.1073/pnas.1106420108 [PubMed: 21873229]
- Choi JM, Kim WC, Lyoo CH, Kang SY, Lee PH, Baik JS, Koh SB, Ma HI, Sohn YH, Lee MS, Kim YJ. Association of mutations in the glucocerebrosidase gene with Parkinson disease in a Korean population. *Neurosci Lett.* 2012; 514(1):12–5. DOI: 10.1016/j.neulet.2012.02.035 [PubMed: 22387070]
- Colom-Cadena M, Gelpi E, Marti MJ, Charif S, Dols-Icardo O, Blesa R, Clarimon J, Lleo A. MAPT H1 haplotype is associated with enhanced alpha-synuclein deposition in dementia with Lewy bodies. *Neurobiol Aging.* 2013; 34(3):936–42. DOI: 10.1016/j.neurobiolaging.2012.06.015 [PubMed: 22819391]
- Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R. Genomes Project Analysis G. The variant call format and VCFtools. *Bioinformatics.* 2011; 27(15):2156–8. DOI: 10.1093/bioinformatics/btr330 [PubMed: 21653522]
- den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat.* 2000; 15(1):7–12. DOI: 10.1002/(SICI)1098-1004(200001)15:1<7::AID-HUMU4>3.0.CO;2-N [PubMed: 10612815]
- Denson MA, Wszolek ZK, Pfeiffer RF, Wszolek EK, Paschall TM, McComb RD. Familial parkinsonism, dementia, and Lewy body disease: study of family G. *Ann Neurol.* 1997; 42(4): 638–43. DOI: 10.1002/ana.410420415 [PubMed: 9382476]
- Duran R, Mencacci NE, Angeli AV, Shuai M, Deas E, Houlden H, Mehta A, Hughes D, Cox TM, Deegan P, Schapira AH, Lees AJ, Limousin P, Jarman PR, Bhatia KP, Wood NW, Hardy J, Foltynie T. The glucocerebrosidase E326K variant predisposes to Parkinson's disease, but does not cause Gaucher's disease. *Mov Disord.* 2013; 28(2):232–6. DOI: 10.1002/mds.25248 [PubMed: 23225227]
- Dvir H, Harel M, McCarthy AA, Toker L, Silman I, Futerman AH, Sussman JL. X-ray structure of human acid-beta-glucosidase, the defective enzyme in Gaucher disease. *EMBO reports.* 2003; 4(7):704–9. DOI: 10.1038/sj.embor.embor873 [PubMed: 12792654]
- Escott-Price V, Nalls MA, Morris HR, Lubbe S, Brice A, Gasser T, Heutink P, Wood NW, Hardy J, Singleton AB, Williams NM. International Parkinson's Disease Genomics C, members I.c. Polygenic risk of Parkinson disease is correlated with disease age at onset. *Ann Neurol.* 2015; 77(4):582–91. DOI: 10.1002/ana.24335 [PubMed: 25773351]
- Federoff M, Jimenez-Rolando B, Nalls MA, Singleton AB. A large study reveals no association between APOE and Parkinson's disease. *Neurobiol Dis.* 2012; 46(2):389–92. DOI: 10.1016/j.nbd.2012.02.002 [PubMed: 22349451]
- Fuchs J, Nilsson C, Kachergus J, Munz M, Larsson EM, Schule B, Langston JW, Middleton FA, Ross OA, Hulihan M, Gasser T, Farrer MJ. Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. *Neurology.* 2007; 68(12):916–22. [PubMed: 17251522]
- Geiger JT, Arthur KC, Dawson TM, Rosenthal LS, Pantelyat A, Albert M, Hillis AE, Crain B, Pletnikova O, Troncoso JC, Scholz SW. C9orf72 Hexanucleotide Repeat Analysis in Cases with Pathologically Confirmed Dementia with Lewy Bodies. *Neurodegener Dis.* 2016; 16(5–6):370–2. DOI: 10.1159/000445872 [PubMed: 27241037]

- Gwinn-Hardy K, Mehta ND, Farrer M, Maraganore D, Muentner M, Yen SH, Hardy J, Dickson DW. Distinctive neuropathology revealed by alpha-synuclein antibodies in hereditary parkinsonism and dementia linked to chromosome 4p. *Acta Neuropathol.* 2000; 99(6):663–72. [PubMed: 10867800]
- Halliday G, Brooks W, Arthur H, Creasey H, Broe GA. Further evidence for an association between a mutation in the APP gene and Lewy body formation. *Neurosci Lett.* 1997; 227(1):49–52. [PubMed: 9178856]
- Hardy J, Crook R, Prihar G, Roberts G, Raghavan R, Perry R. Senile dementia of the Lewy body type has an apolipoprotein E epsilon 4 allele frequency intermediate between controls and Alzheimer's disease. *Neurosci Lett.* 1994; 182(1):1–2. [PubMed: 7891871]
- Hernandez DG, Nalls MA, Moore M, Chong S, Dillman A, Tratzuni D, Gibbs JR, Ryten M, Arepalli S, Weale ME, Zonderman AB, Troncoso J, O'Brien R, Walker R, Smith C, Bandinelli S, Traynor BJ, Hardy J, Singleton AB, Cookson MR. Integration of GWAS SNPs and tissue specific expression profiling reveal discrete eQTLs for human traits in blood and brain. *Neurobiol Dis.* 2012; 47(1):20–8. DOI: 10.1016/j.nbd.2012.03.020 [PubMed: 22433082]
- Huxford T, Huang DB, Malek S, Ghosh G. The crystal structure of the IkappaBalpha/NF-kappaB complex reveals mechanisms of NF-kappaB inactivation. *Cell.* 1998; 95(6):759–70. [PubMed: 9865694]
- Isaacs AM, Johannsen P, Holm I, Nielsen JE, consortium FR. Frontotemporal dementia caused by CHMP2B mutations. *Current Alzheimer research.* 2011; 8(3):246–51. [PubMed: 21222599]
- Ishikawa A, Piao YS, Miyashita A, Kuwano R, Onodera O, Ohtake H, Suzuki M, Nishizawa M, Takahashi H. A mutant PSEN1 causes dementia with Lewy bodies and variant Alzheimer's disease. *Ann Neurol.* 2005; 57(3):429–34. DOI: 10.1002/ana.20393 [PubMed: 15732120]
- Ishikawa A, Takahashi H, Tanaka H, Hayashi T, Tsuji S. Clinical features of familial diffuse Lewy body disease. *Eur Neurol.* 1997; 38(Suppl 1):34–8. [PubMed: 9276199]
- Johnson JO, Mandrioli J, Benatar M, Abramzon Y, Van Deerlin VM, Trojanowski JQ, Gibbs JR, Brunetti M, Gronka S, Wu J, Ding J, McCluskey L, Martinez-Lage M, Falcone D, Hernandez DG, Arepalli S, Chong S, Schymick JC, Rothstein J, Landi F, Wang YD, Calvo A, Mora G, Sabatelli M, Monsurro MR, Battistini S, Salvi F, Spataro R, Sola P, Borghero G, Galassi G, Scholz SW, Taylor JP, Restagno G, Chio A, Traynor BJ, Consortium I. Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron.* 2010; 68(5):857–64. DOI: 10.1016/j.neuron.2010.11.036 [PubMed: 21145000]
- Kamboh MI, Aston CE, Perez-Tur J, Kokmen E, Ferrell RE, Hardy J, DeKosky ST. A novel mutation in the apolipoprotein E gene (APOE*4 Pittsburgh) is associated with the risk of late-onset Alzheimer's disease. *Neurosci Lett.* 1999; 263(2–3):129–32. [PubMed: 10213152]
- Keogh MJ, Kurzawa-Akanbi M, Griffin H, Douroudis K, Ayers KL, Hussein RI, Hudson G, Pyle A, Cordell HJ, Attems J, McKeith IG, O'Brien JT, Burn DJ, Morris CM, Thomas AJ, Chinnery PF. Exome sequencing in dementia with Lewy bodies. *Translational psychiatry.* 2016; 6:e728.doi: 10.1038/tp.2015.220 [PubMed: 26836416]
- Kim WS, Kagedal K, Halliday GM. Alpha-synuclein biology in Lewy body diseases. *Alzheimers Res Ther.* 2014; 6(5):73.doi: 10.1186/s13195-014-0073-2 [PubMed: 25580161]
- Lantos PL, Ovenstone IM, Johnson J, Clelland CA, Roques P, Rossor MN. Lewy bodies in the brain of two members of a family with the 717 (Val to Ile) mutation of the amyloid precursor protein gene. *Neurosci Lett.* 1994; 172(1–2):77–9. [PubMed: 8084541]
- Leverenz JB, Fishel MA, Peskind ER, Montine TJ, Nochlin D, Steinbart E, Raskind MA, Schellenberg GD, Bird TD, Tsuang D. Lewy body pathology in familial Alzheimer disease: evidence for disease- and mutation-specific pathologic phenotype. *Arch Neurol.* 2006; 63(3):370–6. DOI: 10.1001/archneur.63.3.370 [PubMed: 16533963]
- Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2009; 25(14):1754–60. DOI: 10.1093/bioinformatics/btp324 [PubMed: 19451168]
- Lippa CF, Duda JE, Grossman M, Hurtig HI, Aarsland D, Boeve BF, Brooks DJ, Dickson DW, Dubois B, Emre M, Fahn S, Farmer JM, Galasko D, Galvin JE, Goetz CG, Growdon JH, Gwinn-Hardy KA, Hardy J, Heutink P, Iwatsubo T, Kosaka K, Lee VM, Leverenz JB, Masliah E, McKeith IG, Nussbaum RL, Olanow CW, Ravina BM, Singleton AB, Tanner CM, Trojanowski JQ, Wszolek ZK, Group DPW. DLB and PDD boundary issues: diagnosis, treatment, molecular pathology, and

biomarkers. *Neurology*. 2007; 68(11):812–9. DOI: 10.1212/01.wnl.0000256715.13907.d3 [PubMed: 17353469]

- Malini E, Grossi S, Deganuto M, Rosano C, Parini R, Dominisini S, Cariati R, Zampieri S, Bembi B, Filocamo M, Dardis A. Functional analysis of 11 novel GBA alleles. *Eur J Hum Genet*. 2014; 22(4):511–6. DOI: 10.1038/ejhg.2013.182 [PubMed: 24022302]
- McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, Cummings J, Duda JE, Lippa C, Perry EK, Aarsland D, Arai H, Ballard CG, Boeve B, Burn DJ, Costa D, Del Ser T, Dubois B, Galasko D, Gauthier S, Goetz CG, Gomez-Tortosa E, Halliday G, Hansen LA, Hardy J, Iwatsubo T, Kalara RN, Kaufer D, Kenny RA, Korczyn A, Kosaka K, Lee VM, Lees A, Litvan I, Londos E, Lopez OL, Minoshima S, Mizuno Y, Molina JA, Mukaetova-Ladinska EB, Pasquier F, Perry RH, Schulz JB, Trojanowski JQ, Yamada M. Consortium on DLB. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology*. 2005; 65(12):1863–72. [PubMed: 16237129]
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010; 20(9):1297–303. DOI: 10.1101/gr.107524.110 [PubMed: 20644199]
- Meeus B, Verstraeten A, Crosiers D, Engelborghs S, Van den Broeck M, Mattheijssens M, Peeters K, Corsmit E, Elinck E, Pickut B, Vandenbergh R, Cras P, De Deyn PP, Van Broeckhoven C, Theuns J. DLB and PDD: a role for mutations in dementia and Parkinson disease genes? *Neurobiol Aging*. 2012; 33(3):629, e5–e18. DOI: 10.1016/j.neurobiolaging.2011.10.014 [PubMed: 22118943]
- Muratore CR, Rice HC, Srikanth P, Callahan DG, Shin T, Benjamin LN, Walsh DM, Selkoe DJ, Young-Pearse TL. The familial Alzheimer's disease APPV717I mutation alters APP processing and Tau expression in iPSC-derived neurons. *Hum Mol Genet*. 2014; 23(13):3523–36. DOI: 10.1093/hmg/ddu064 [PubMed: 24524897]
- Nalls MA, Duran R, Lopez G, Kurzawa-Akanbi M, McKeith IG, Chinnery PF, Morris CM, Theuns J, Crosiers D, Cras P, Engelborghs S, De Deyn PP, Van Broeckhoven C, Mann DM, Snowden J, Pickering-Brown S, Halliwell N, Davidson Y, Gibbons L, Harris J, Sheerin UM, Bras J, Hardy J, Clark L, Marder K, Honig LS, Berg D, Maetzler W, Brockmann K, Gasser T, Novellino F, Quattrone A, Annesi G, De Marco EV, Rogaeva E, Masellis M, Black SE, Bilbao JM, Foroud T, Ghetti B, Nichols WC, Pankratz N, Halliday G, Lesage S, Klebe S, Durr A, Duyckaerts C, Brice A, Giasson BI, Trojanowski JQ, Hurtig HI, Tayebi N, Landazabal C, Knight MA, Keller M, Singleton AB, Wolfsberg TG, Sidransky E. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. *JAMA Neurol*. 2013; 70(6):727–35. DOI: 10.1001/jamaneurol.2013.1925 [PubMed: 23588557]
- Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res*. 2003; 31(13):3812–4. [PubMed: 12824425]
- Nichols WC, Pankratz N, Marek DK, Pauciulo MW, Elsaesser VE, Halter CA, Rudolph A, Wojcieszek J, Pfeiffer RF, Foroud T. Parkinson Study Group PI. Mutations in GBA are associated with familial Parkinson disease susceptibility and age at onset. *Neurology*. 2009; 72(4):310–6. DOI: 10.1212/01.wnl.0000327823.81237.d1 [PubMed: 18987351]
- Ohara K, Takauchi S, Kokai M, Morimura Y, Nakajima T, Morita Y. Familial dementia with Lewy bodies (DLB). *Clin Neuropathol*. 1999; 18(5):232–9. [PubMed: 10505432]
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007; 81(3):559–75. [PubMed: 17701901]
- Rambaldi D, Ciccarelli FD. FancyGene: dynamic visualization of gene structures and protein domain architectures on genomic loci. *Bioinformatics*. 2009; 25(17):2281–2. DOI: 10.1093/bioinformatics/btp381 [PubMed: 19542150]
- Reitz C, Brayne C, Mayeux R. Epidemiology of Alzheimer disease. *Nat Rev Neurol*. 2011; 7(3):137–52. DOI: 10.1038/nrneurol.2011.2 [PubMed: 21304480]
- Rogaeva EA, Fafel KC, Song YQ, Medeiros H, Sato C, Liang Y, Richard E, Rogaev EI, Frommelt P, Sadovnick AD, Meschino W, Rockwood K, Boss MA, Mayeux R, St George-Hyslop P. Screening for PS1 mutations in a referral-based series of AD cases: 21 novel mutations. *Neurology*. 2001; 57(4):621–5. [PubMed: 11524469]

- Rozenberg R, Fox DC, Sobreira E, Pereira LV. Detection of 12 new mutations in Gaucher disease Brazilian patients. *Blood Cells Mol Dis.* 2006; 37(3):204–9. DOI: 10.1016/j.bcmd.2006.09.004 [PubMed: 17059888]
- Sailer A, Scholz SW, Gibbs JR, Tucci A, Johnson JO, Wood NW, Plagnol V, Hummerich H, Ding J, Hernandez D, Hardy J, Federoff HJ, Traynor BJ, Singleton AB, Houlden H. Exome sequencing in an SCA14 family demonstrates its utility in diagnosing heterogeneous diseases. *Neurology.* 2012; 79(2):127–31. DOI: 10.1212/WNL.0b013e31825f048e [PubMed: 22675081]
- Scholz SW, Bras J. Genetics Underlying Atypical Parkinsonism and Related Neurodegenerative Disorders. *International journal of molecular sciences.* 2015; 16(10):24629–55. DOI: 10.3390/ijms161024629 [PubMed: 26501269]
- Schulte EC, Fukumori A, Mollenhauer B, Hor H, Arzberger T, Perneczky R, Kurz A, Diehl-Schmid J, Hull M, Lichtner P, Eckstein G, Zimprich A, Haubenberger D, Pirker W, Brucke T, Bereznaï B, Molnar MJ, Lorenzo-Betancor O, Pastor P, Peters A, Gieger C, Estivill X, Meitinger T, Kretschmar HA, Trenkwalder C, Haass C, Winkelmann J. Rare variants in beta-Amyloid precursor protein (APP) and Parkinson's disease. *Eur J Hum Genet.* 2015; 23(10):1328–33. DOI: 10.1038/ejhg.2014.300 [PubMed: 25604855]
- Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nature methods.* 2014; 11(4):361–2. DOI: 10.1038/nmeth.2890 [PubMed: 24681721]
- Shulman JM, De Jager PL, Feany MB. Parkinson's disease: genetics and pathogenesis. *Annu Rev Pathol.* 2011; 6:193–222. DOI: 10.1146/annurev-pathol-011110-130242 [PubMed: 21034221]
- Sidransky E. Gaucher disease: complexity in a “simple” disorder. *Mol Genet Metab.* 2004; 83(1–2):6–15. DOI: 10.1016/j.ymgme.2004.08.015 [PubMed: 15464415]
- Sidransky E, Lopez G. The link between the GBA gene and parkinsonism. *Lancet Neurol.* 2012; 11(11):986–98. DOI: 10.1016/S1474-4422(12)70190-4 [PubMed: 23079555]
- Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, Bar-Shira A, Berg D, Bras J, Brice A, Chen CM, Clark LN, Condroyer C, De Marco EV, Durr A, Eblan MJ, Fahn S, Farrer MJ, Fung HC, Gan-Or Z, Gasser T, Gershoni-Baruch R, Giladi N, Griffith A, Gurevich T, Januario C, Kropp P, Lang AE, Lee-Chen GJ, Lesage S, Marder K, Mata IF, Mirelman A, Mitsui J, Mizuta I, Nicoletti G, Oliveira C, Ottman R, Orr-Urtreger A, Pereira LV, Quattrone A, Rogaeva E, Rolfs A, Rosenbaum H, Rozenberg R, Samii A, Samadddar T, Schulte C, Sharma M, Singleton A, Spitz M, Tan EK, Tayebi N, Toda T, Troiano AR, Tsuji S, Wittstock M, Wolfsberg TG, Wu YR, Zabetian CP, Zhao Y, Ziegler SG. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med.* 2009; 361(17):1651–61. [PubMed: 19846850]
- Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muentner M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K. alpha-Synuclein locus triplication causes Parkinson's disease. *Science.* 2003; 302(5646):841. [PubMed: 14593171]
- Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, Buchman AS, Larson EB, Crane PK, Kaye JA, Kramer P, Woltjer R, Trojanowski JQ, Weintraub D, Chen-Plotkin AS, Irwin DJ, Rick J, Schellenberg GD, Watson GS, Kukull W, Nelson PT, Jicha GA, Neltner JH, Galasko D, Masliah E, Quinn JF, Chung KA, Yearout D, Mata IF, Wan JY, Edwards KL, Montine TJ, Zabetian CP. APOE epsilon4 increases risk for dementia in pure synucleinopathies. *JAMA Neurol.* 2013; 70(2):223–8. DOI: 10.1001/jamaneurol.2013.600 [PubMed: 23407718]
- van Blitterswijk M, van Es MA, Hennekam EA, Dooijes D, van Rheeën W, Medic J, Bourque PR, Schelhaas HJ, van der Kooi AJ, de Visser M, de Bakker PI, Veldink JH, van den Berg LH. Evidence for an oligogenic basis of amyotrophic lateral sclerosis. *Hum Mol Genet.* 2012; 21(17):3776–84. DOI: 10.1093/hmg/dds199 [PubMed: 22645277]
- Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 2010; 38(16):e164.doi: 10.1093/nar/gkq603 [PubMed: 20601685]
- Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atares B, Llorens V, Gomez Tortosa E, del Ser T, Munoz DG, de Yébenes JG. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol.* 2004; 55(2):164–73. [PubMed: 14755719]

Zimprich A, Muller-Myhsok B, Farrer M, Leitner P, Sharma M, Hulihan M, Lockhart P, Strongosky A, Kachergus J, Calne DB, Stoessl J, Uitti RJ, Pfeiffer RF, Trenkwalder C, Homann N, Ott E, Wenzel K, Asmus F, Hardy J, Wszolek Z, Gasser T. The PARK8 locus in autosomal dominant parkinsonism: confirmation of linkage and further delineation of the disease-containing interval. *Am J Hum Genet.* 2004; 74(1):11–9. [PubMed: 14691730]

Highlights

- *APOE ε4* significantly increases risk for DLB and decreases survival
- Pathogenic missense mutations and risk variants in *GBA*, *PSEN1* and *APP* are common in DLB
- Genetic defects place DLB along a spectrum between Parkinson disease and Alzheimer dementia

Figure 1. Missense mutations in definite DLB cases

Panel **(a)** shows the position of causative mutations and coding risk variants relative to the respective gene and protein sequences. Panel **(b)** illustrates the position of each mutated amino acid residue relative to the 3-D protein or domain structure.

Table 1
Genetic variants implicated in DLB identified by comprehensive genetic analysis of 111 definite DLB cases

Interpretation	Gene	cDNA Change	Protein Change	SNP-ID	Position (hg19)	Accession	SIFT	PolyPhen2 Prediction	MutationTaster Prediction	DLB Exomes			MAF	DLB Exomes		ExAC [†] (EUR)
	<i>GBA</i>	c.1342G>C	p.D448H	rs1064651	1:155,205,518	NM_000157	Tolerated	Benign	Disease-causing	0.00450	0.00000	0.00000	0.00013			
	<i>GBA</i>	c.1226A>G	p.N409S	rs76763715	1:155,205,634	NM_000157	Damaging	Possibly Damaging	Disease-causing	0.01802	0.00225	0.00225	0.00340			
	<i>GBA</i>	c.1093G>A	p.E365K	rs2230288	1:155,206,167	NM_000157	Tolerated	Benign	Disease-causing	0.03604	0.00901	0.00901	0.01481			
	<i>GBA</i>	c.887G>A	p.R296Q	rs78973108	1:155,207,244	NM_000157	Damaging	Probably Damaging	Disease-causing	0.00450	0.00000	0.00000	0.00004			
	<i>GBA</i>	c.260G>A	p.R87Q	rs78769774	1:155,209,724	NM_000157	Tolerated	Probably Damaging	Disease-causing	0.00450	0.00000	0.00000	-			
	<i>PSEN1</i>	c.617G>C	p.G206A	rs63750082	14:73,659,420	NM_000021	Damaging	Probably Damaging	Disease-causing	0.00450	0.00000	0.00000	-			
	<i>APP</i>	c.2149G>A	p.V717I	rs63750264	21:27,264,096	NM_000484	Tolerated	Probably Damaging	Disease-causing	0.00450	0.00000	0.00000	-			
	<i>APOE^{ε4}</i>	c.388T>C	p.C130R	rs429358	19:45,411,941	NM_000041	Tolerated	Benign	Polymorphism	0.20183	0.14412	0.14412	0.21281			
	<i>APOE^{ε4}</i>	c.526C>T	p.R176C	rs7412	19:45,412,079	NM_000041	Damaging	Probably Damaging	Disease-causing	0.19820	0.09615	0.09615	0.10560			
	<i>APP</i>	c.1795G>A	p.E599K	rs140304729	21:27,284,167	NM_000484	Tolerated	Probably Damaging	Disease-causing	0.00450	0.00000	0.00000	0.00205			
	<i>PSEN1</i>	c.953A>G	p.E318G	rs17125721	14:73,673,178	NM_000021	Damaging	Benign	Disease-causing	0.04505	0.02252	0.02252	0.02231			

in the DLB genes *APP*, *APOE*, *GBA*, *LRRK2*, *MAPT*, *PSEN1*, *PSEN2*, *SCARB2* and *SNCA* were investigated in 111 definite DLB cases.
Disease-associated mutations, their predicted effects and minor allele frequencies. No pathogenic missense mutations were detected in *LRRK2*, *MAPT*, *PSEN2*, *SCARB2* and *SNCA*.

on Consortium (ExAC) version 3; minor allele frequencies for individuals of European descent are shown

shown are part of the *APOE ε4* risk allele

allele frequency

Table 2
Genetic, clinical and pathological characteristics of definite DLB cases carrying pathogenic mutations or risk variants

Patient ID	Genetic Characteristics			Clinical Characteristics			Pathological Characteristics					
	Mutation(s) or Risk Variants	APOE Status	Clinical Diagnosis	Age at Onset	Age at Death	Sex	Family History [†]	Primary Diagnosis	DLB Subtype	AD Pathology Level	CERAD Score	BRAAK Stage
1	<i>GBA</i> p.R87Q +/-	e 3/e 4	PDD	53	63	M	Positive	DLB	Neocortical	Intermediate	C	3
2	<i>GBA</i> p.R296Q +/-	e 4/e 4	DLB	66	78	M	-	DLB	Neocortical	High	B	6
3	<i>GBA</i> p.D448H +/-	e 2/e 3	PDD	68	81	F	Positive	DLB	Limbic	Low	0	2
4	<i>GBA</i> p.E365K +/-	e 3/e 3	PDD	69	82	F	Positive	DLB	Neocortical	Intermediate	B	2
5	<i>GBA</i> p.E365K +/-	e 3/e 4	DLB	49	57	M	Positive	DLB	Neocortical	High	C	6
6	<i>GBA</i> p.E365K +/-	e 3/e 4	PDD	58	73	F	-	DLB	Neocortical	Low	0	3
7	<i>GBA</i> p.E365K +/-	e 3/e 3	PDD	51	84	F	-	DLB	Neocortical	Not	0	4
8	<i>GBA</i> p.E365K +/-	e 3/e 3	PDD	64	75	M	-	DLB	Neocortical	Intermediate	B	2
9	<i>GBA</i> p.E365K +/-	e 3/e 3	PDD	78	85	F	-	DLB	Neocortical	Not	0	4
10	<i>GBA</i> p.E365K +/-	e 4/e 4	PD, AD	43	60	F	Positive	DLB	Neocortical	Not	0	4
11	<i>GBA</i> p.N409S +/-	e 3/e 3	Dementia	79	89	F	-	DLB	Neocortical	Intermediate	B	4
12	<i>GBA</i> p.N409S +/-	e 2/e 3	PDD	58	68	M	-	DLB	Neocortical	Not	0	0
13	<i>GBA</i> p.N409S +/-	e 3/e 3	PDD	43	65	M	Positive	DLB	Neocortical	Low	C	2
14	<i>GBA</i> p.N409S +/-	e 3/e 3	PDD	40	73	M	-	DLB	Neocortical	Not	0	0
15	<i>PSEN1</i> p.E318G +/-	e 3/e 3	PDD	55	70	F	-	DLB	Limbic	Intermediate	B	4
16	<i>PSEN1</i> p.E318G +/-	e 3/e 4	AD	75	85	F	-	DLB	Neocortical	High	C	5
17	<i>PSEN1</i> p.E318G +/-	e 3/e 3	PDD	76	84	M	-	DLB	Neocortical	Low	0	4

Patient ID	Genetic Characteristics			Clinical Characteristics			Pathological Characteristics					
	Mutation(s) or Risk Variants	APOE Status	Clinical Diagnosis	Age at Onset	Age at Death	Sex	Family History [†]	Primary Diagnosis	DLB Subtype	AD Pathology Level	CERAD Score	BRAAK Stage
8	<i>PSEN1</i> p.E318G +/-	e 3/e 3	PDD	55	81	M	-	DLB	Neocortical	Intermediate	B	3
9	<i>PSEN1</i> p.E318G +/-	e 3/e 3	AD	65	71	M	-	DLB	Neocortical	Intermediate	C	4
10	<i>PSEN1</i> p.G206A +/-	e 3/e 3	AD	56	64	F	Positive	DLB	Neocortical	High	C	6
11	<i>PSEN1</i> p.E318G +/-	e 3/e 3	PDD	67	85	M	-	DLB	Neocortical	Low	A	2
12	<i>PSEN1</i> p.E318G +/-	e 3/e 3	FTD, PD	82	91	M	Positive	DLB	Neocortical	High	B	6
13	<i>PSEN1</i> p.E318G +/-	e 3/e 3	PDD	76	87	M	-	DLB	Neocortical	Intermediate	B	4
14	<i>PSEN1</i> p.E318G +/-	e 3/e 4	FTD	59	64	F	Positive	DLB	Neocortical	High	C	6
15	<i>APP</i> p.E599K +/-	e 3/e 3	PDD	62	84	M	Positive	DLB	Limbic	Intermediate	B	3
16	<i>APP</i> p.V717I +/-	e 3/e 3	DLB, AD	59	71	F	Positive	DLB	Neocortical	High	C	6

Shown are the genetic, clinical and pathological characteristics of DLB cases carrying disease-associated missense mutations in *GBA*, *PSEN1* and *APP*.

Abbreviations: AD, Alzheimer dementia; DLB, dementia with Lewy bodies; PDD, Parkinson disease dementia; FTD, frontotemporal dementia, +/- denotes heterozygous mutation carrier

Family history was positive if the patient reported at least one first- or second-degree relative with a diagnosis of dementia, cognitive impairment, parkinsonism, Dementia with Lewy bodies, Parkinson disease, or Alzheimer dementia