

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

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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 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* $\epsilon 4$ 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.

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 ε4* 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 (Qiagen, 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* $\epsilon 4$ 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* $\epsilon 4$ 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* $\epsilon 4$ risk allele was significantly higher in our DLB cohort compared to Caucasian controls (p -value < 0.001) and survival was significantly shorter in *APOE* $\epsilon 4$ 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* $\epsilon 4$ 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* $\epsilon 4$ 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.

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Figure Legends

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

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.