Neurobiology of Disease 94 (2016) 55–62

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

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

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ARTICLE INFO

Article history:
Received 22 February 2016
Revised 8 June 2016
Accepted 11 June 2016
Available online 14 June 2016

Keywords:
Dementia with Lewy bodies
Exome sequencing
Alzheimer dementia
Lewy body dementia
GBA
PSEN1
APP
APOE

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 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.

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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

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Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2016.06.004
0969-9961/Published by Elsevier Inc.

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 Fig. 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, Fig. 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