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Genetic analysis of neurodegenerative diseases in a pathology cohort

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ABSTRACT

Molecular genetic research provides unprecedented opportunities to examine genotype-phenotype correlations underlying complex syndromes. To investigate pathogenic mutations and genotype-phenotype relationships in diverse neurodegenerative conditions, we performed a rare variant analysis of damaging mutations in autopsy-confirmed neurodegenerative cases from the Johns Hopkins Brain Resource Center (n = 1243 patients). We used NeuroChip genotyping and *C9orf72* hexanucleotide repeat analysis to rapidly screen our cohort for disease-causing mutations. In total, we identified 42 individuals who carried a pathogenic mutation in *LRRK2, GBA, APP, PSEN1, MAPT, GRN, C9orf72, SETX, SPAST*, or *CSF1R*, and we provide a comprehensive description of the diverse clinicopathological features of these well-characterized cases. Our study highlights the utility of high-throughput genetic screening arrays to establish a molecular diagnosis in individuals with complex neurodegenerative syndromes, to broaden disease phenotypes and to provide insights into unexpected disease associations.

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1. Introduction

Age-related neurodegenerative diseases are a growing public health concern because of population aging within developed societies. For example, the U.S. population over 65 years of age will nearly double by 2050, and the number of people with Alzheimer's disease is projected to dramatically increase (Hebert et al., 2013; Ortman et al., 2014). There is a critical need to develop disease-modifying treatments to ameliorate these age-related diseases. Improved understanding of the pathogenic molecular defects that lead to neurodegeneration is crucial for achieving this goal.

Until recently, systematic screening of disease-causing mutations was prohibitively expensive and cumbersome. This has hampered the use of genetic testing in the clinic, where the standard approach is to screen only a small number of mutations in

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patients with a clear family history and distinct phenotypes. Recent advances in high-throughput genotyping technologies are changing this approach, as they allow us to rapidly screen thousands of disease-associated genetic variants at low cost for diverse clinical and scientific applications. It is now feasible to broadly apply this type of genetic screening to large patient populations to study the diverse genotype-phenotype correlations and to identify unexpected disease associations.

To illustrate this point, we genotyped 1243 pathologically confirmed neurodegenerative disease cases using the NeuroChip, an inexpensive, off-the-shelf genotyping array that rapidly detects mutations and genetic risk variants previously implicated in neurological disease (Blauwendraat et al., 2017). We chose this autopsy-based patient series as it provides diagnostic certainty and avoids the confusion that may arise from mimic syndromes in clinically heterogeneous neurodegenerative conditions.

Analyzing these high-yield data for disease-causing mutations, we identified a diverse spectrum of mutation carriers in this well-characterized cohort and observed novel genotype-phenotype correlations. For example, we provide compelling evidence that the pathogenic *LRRK2* G2019S mutation not only causes Parkinson's disease, but may also present with pathological changes consistent with progressive supranuclear palsy (PSP). Our data established a molecular diagnosis in several patients where the clinical and pathological diagnosis gave disparate results and led to the identification of a molecular cause in cases with unclassified neurodegenerative conditions. Importantly, we have created a resource of genomic findings in pathologically defined neurodegenerative diseases. We expect the number of genes related to neurodegeneration to grow over time, and other researchers can freely access our pathologically and genetically characterized cohort to replicate and extend their findings.

2. Materials and methods

2.1. Samples

Tissue samples were obtained from the Johns Hopkins Brain Resource Center, which encompasses the Johns Hopkins Morris K. Udall Center of Excellence for Parkinson's Disease Research, the Johns Hopkins Alzheimer's Disease Research Center, and autopsy cases from the Baltimore Longitudinal Study on Aging. Patients included in this cohort were referred by clinical and research centers at Johns Hopkins University and across academic centers in Maryland, USA. All participants gave written informed consent for postmortem brain donation. A total of 1243 neurodegenerative disease cases were selected for genotyping (Table 1 shows a detailed summary of the study cohort) using the following inclusion/exclusion criteria: 1) each case had a pathological diagnosis of a neurodegenerative syndrome, 2) had no history of a primary or secondary CNS malignancy, and 3) no known genetic cause of disease.

The cohort included the following neurodegenerative disease entities: Alzheimer's disease, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), hippocampal sclerosis, Lewy body dementia (LBD), multiple system atrophy (MSA), Parkinson's disease, PSP, corticobasal degeneration, as well as unclassified or rare neurodegenerative diseases. Individuals with more than one neurodegenerative disease, for example, Alzheimer's disease plus LBD, were labeled as "complex" cases (Fig. 1).

2.2. Genotyping

DNA was extracted from frozen brain tissue using phenol-chloroform extraction and diluted in TE buffer (Qiagen, Hilden, Germany). Genotyping was performed using the NeuroChip (Illumina, San Diego, CA, USA), a microarray that comprised a tagging variant backbone (n = 306,670 variants) and 179,467 variants of custom "neuro" content. This chip is designed for rapid, comprehensive, and affordable screening of known single nucleotide variants implicated in diverse neurodegenerative diseases. Variants included on this platform were reported in the Human Gene Mutation Database (HGMD Professional 2016.4, QIAGEN), the NHGRI GWAS Catalog (www.ebi.ac.uk/gwas/), the Parkinson's Disease Mutation Database (www.molgen.vib-ua.be/PDMutDB), the Alzheimer's Disease and Frontotemporal Dementia Database (www.molgen.ua.ac.be/admutations/), the Online Mendelian Inheritance in Man (OMIM) database (www.ncbi.nlm.nih.gov/omim/), literature review as well as ongoing research studies in neurodegenerative diseases. The detailed content of this versatile genotyping array has been described elsewhere (Blauwendraat et al., 2017). NeuroChip genotyping was carried out as per the manufacturer's instructions (Illumina). Briefly, for each sample, a total of 250 ng of high-quality genomic DNA was amplified and enzymatically fragmented. The resulting fragments were alcohol-precipitated and resuspended in buffer. Next, the fragmented DNA solution was

Table 1
Johns Hopkins brain bank cohort description

Disease category		Sample characteristics		
Pathological diagnosis	No. of cases	Age at death (mean, ± SD)	Sex (% female)	Race (% white)
Alzheimer's disease (AD)	624	82 (±11)	62%	96%
Complex (more than one disease)				
AD + Lewy body disease (PD, LBD)	216	79 (±09)	41%	98%
AD + ALS-FTD	27	80 (±10)	56%	100%
AD + Atypical parkinsonism	26	78 (±11)	38%	96%
Other complex cases	40	79 (±13)	45%	98%
Lewy body disease				
PD	50	77 (±10)	32%	98%
LBD	49	78 (±07)	24%	96%
ALS-FTD	84	65 (±13)	45%	100%
Atypical parkinsonism				
MSA	13	67 (±12)	58%	100%
PSP	37	75 (±07)	42%	95%
CBD	7	73 (±08)	57%	100%
Other neurodegenerative disease				
Rare neurodegenerative syndromes	9	68 (±14)	33%	100%
Unclassified neurodegenerative cases	47	78 (±23)	49%	98%
Hippocampal sclerosis	14	79 (±11)	50%	100%
Total	**1243**	**79 (±12)**	**52%**	**97%**

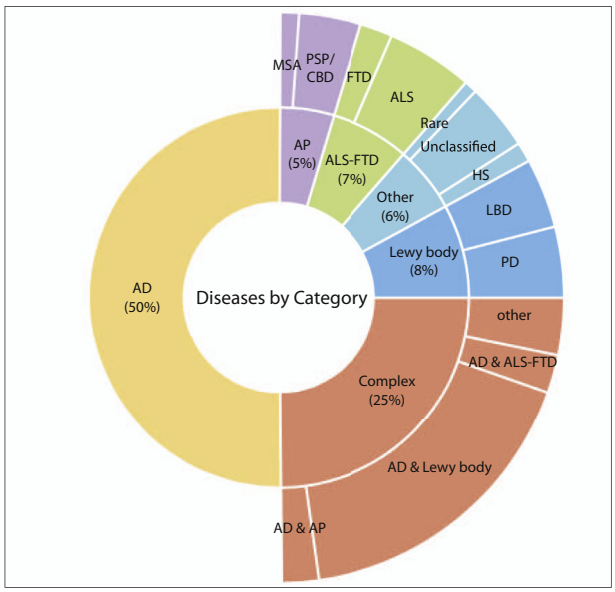

Fig. 1. This multilayer pie chart illustrates the composition of the Johns Hopkins brain bank cohort by disease category. Abbreviations: AD, Alzheimer's dementia; ALS, amyotrophic lateral sclerosis; AP, atypical parkinsonism; CBD, corticobasal degeneration; FTD, frontotemporal dementia; HS, hippocampal sclerosis; LBD, Lewy body dementia; MSA, multiple system atrophy; PD, Parkinson's disease; PSP, progressive supranuclear palsy.

hybridized to the NeuroChip array using a Tecan Freedom EVO liquid-handling robot (Tecan, Research Triangle Park, NC). After hybridization, automated allele-specific, enzymatic base extension and fluorophore staining were performed. The stained genotyping arrays were washed, sealed, and vacuum-dried before scanning them on the Illumina High-Scan system. Raw data files were imported into GenomeStudio (version 2.0, Genotyping Module, Illumina) using a custom-generated sample sheet, and genotypes were called using a GenCall threshold of 0.15. The resulting genotype data were exported in a ped file format using the Illumina-to-PLINK module (version 2.1.4).

2.3. Quality control

Quality control was conducted in PLINK (version 1.9) (Chang et al., 2015). Of the 1243 genotyped samples, 3 were excluded because of a low call rate of <98%, 4 individuals were removed because of ambiguous gender, and one Alzheimer's disease case with Down syndrome was genotyped in error and excluded from the analysis. In total, 1235 samples passed sample quality control and were evaluated for pathogenic mutations. An illustration showing the analysis workflow is shown in Figure 2.

2.4. Rare variant analysis and variant validation

Monogenic diseases due to highly penetrant rare mutations are especially informative for genotype-phenotype correlations as they can be attributed to well-defined molecular defects. To identify such damaging mutations in our brain bank cohort, we conducted a rare variant analysis by filtering our data by the professionally curated Human Gene Mutation Database content (HGMD Professional, 2016.4) included on the NeuroChip (n = 8086 disease-associated variants) (Blauwendraat et al., 2017). Taking into account that disease-causing mutations are rare because of selective pressure, we next excluded variants with a minor allele frequency >0.005 using population frequency estimates from ANNOVAR

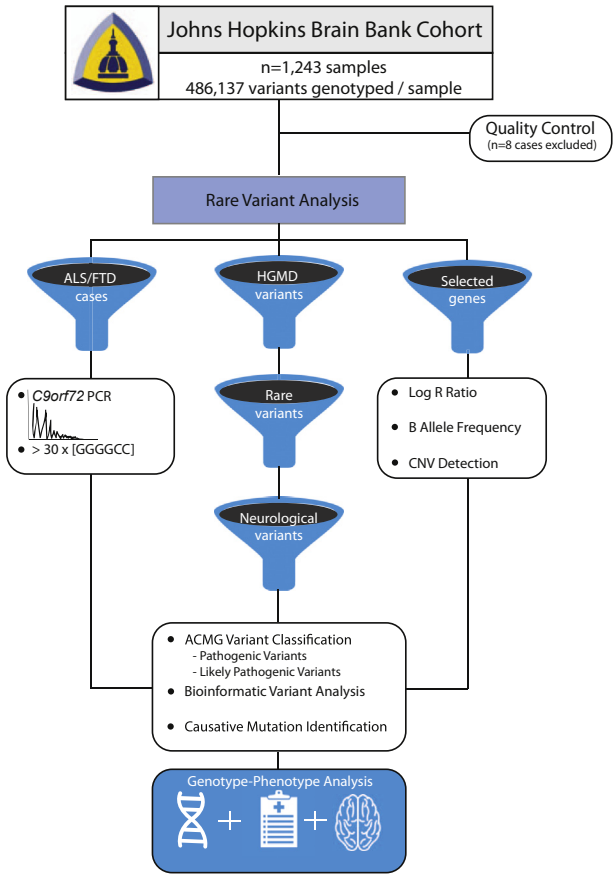

Fig. 2. Illustration showing an overview of the analysis workflow that we applied to identify patients with pathogenic mutations, including filtering the data to extract rare, damaging neurological mutations in the Human Genome Mutation Database content, performing C9orf72 repeat expansion screening in pathologically confirmed ALS/FTD patients, and a copy number variant analysis of selected genes. Abbreviations: ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; HGMD, Human Gene Mutation Database.

(Wang et al., 2010) and variants with a maximum population frequency exceeding 50 alleles according to ExAC (version 0.3.1) (Lek et al., 2016), reducing the data set to 446 variants. To rule out possible genotyping errors of the identified rare HGMD variants, we reviewed GenTrain scores and visualized Cartesian plots of genotype clusters in GenomeStudio (version 2.0, Genotyping Module, Illumina). Genotypes with borderline GenTrain scores (<0.7), variants with ambiguous genotype cluster separation, and multiallelic variants were selected for validation using bidirectional Sanger sequencing using Big-Dye Terminator v.3.1 sequencing chemistry (Applied Biosystems Inc., Foster City, CA, USA). Sequence reactions were run on an ABI 3730xl genetic analyzer and interpreted using Geneious software (version 10.0.7, Biomatters Ltd, Newark, NJ, USA). This validation step identified 10 variants that were not confirmed and excluded from the study. Next, we obtained pathogenicity prediction annotations (SIFT, PolyPhen-2, M-CAP, CADD) and nucleotide conservation predictions (GERP++) from ANNOVAR for all remaining rare HGMD variants (Adzhubei et al., 2010; Davydov et al., 2010; Jagadeesh et al., 2016; Kircher et al., 2014; Ng and Henikoff, 2003; Wang et al., 2010). We then reviewed all these variants for known association with neurological disease (n = 328 variants). After exclusion of variants that were associated with non-neurological human traits/diseases, we determined variants according to ACMG consensus guidelines and focused on pathogenic and likely pathogenic variants that are presumed to be disease-causing (Richards et al., 2015). This step identified 21

missense mutations in 1 or more patients located within the genes *LRRK2, GBA, APP, PSEN1, MAPT, GRN, SETX, SPAST,* and *CSF1R* (Supplementary Table 1). Owing to the fact that *GBA* has a pseudogene with high homology to the functional gene that may lead to false-positive genotype calls, we performed validation experiments of all *GBA* variants using a Sanger sequencing method described elsewhere (Stone et al., 2000). This step identified one GBA variant (R159W) that was not confirmed and therefore excluded.

2.5. C9orf72 *hexanucleotide repeat expansion screening*

C9orf72 hexanucleotide repeat expansion is the most common, monogenic cause of ALS/FTD. Although the NeuroChip readily detects the previously described Finnish risk haplotype surrounding the *C9orf72* locus (Laaksovirta et al., 2010), this haplotype is also commonly present in the control population and does not serve as a reliable proxy marker for identifying repeat expansion carriers. We therefore screened all ALS/FTD cases (n = 84) for pathogenic hexanucleotide repeat expansions using repeat-primed PCR as described elsewhere (Renton et al., 2011). The resulting PCR amplicons were analyzed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Grand Island, NY, USA). Expansions of >30 GGGGCC repeats were interpreted as pathogenic.

2.6. *Copy number variant analysis*

Multiplications of *SNCA* and homozygous or compound heterozygous copy number variation in *PARK2* can rarely cause familial Parkinson's disease (Fuchs et al., 2007; Kitada et al., 1998; Lucking et al., 1998; Singleton et al., 2003). Likewise, *APP* duplication or *PSEN1* exon 9 deletion have been demonstrated as rare Mendelian causes of early-onset Alzheimer's disease (Rovelet-Lecrux et al., 2006; Smith et al., 2001). Hence, we undertook a copy number variant analysis of these 4 genes using standard protocols described elsewhere (Matarin et al., 2008). Briefly, B-allele frequency and LogR ratio plots were constructed from GenomeStudio for each of the 4 gene loci ± 500,000 bp flanking regions and visually inspected for gene multiplications or deletions. Pathogenic copy number variants identified in this analysis were confirmed by quantitative PCR.

2.7. *Genotype-phenotype analysis*

Medical charts, pathology reports and histopathology sections of causative mutation carriers were reviewed and summarized by neuropathologists JCT and OP and board-certified neurologist SWS.

3. Results

3.1. *Rare variant analysis*

We performed genomewide genotyping of 1243 samples diagnosed with neurodegenerative diseases obtained from the Johns Hopkins Brain Bank using the NeuroChip array. This platform provided information for 468,137 variants/case, of which 8086 variants are reported in the Human Gene Mutation Database, a collection of disease-associated genetic variants.

Using these samples, we identified a total of 42 patients (approximately 3.4% of the total cohort) carrying a damaging mutation in one or more of the following genes: 1) *LRRK2*, 2) *GBA*, 3) *APP*, 4) *PSEN1*, 5) *MAPT*, 6) *GRN*, 7) *C9orf72*, 8) *SETX*, 9) *SPAST,* and 10) *CSF1R*. Clinical and pathological features of these individuals are shown in Table 2 and more detailed case-by-case descriptions are provided in the Supplementary Materials. We summarize the genetic characteristics, bioinformatic modeling data, and the pathogenicity predictions of the identified mutations in Supplementary Table 1. In the following, we describe each of the mutation carriers categorized by gene, highlighting novel observations that arise from our data.

3.1.1. LRRK2 *mutations*

We identified 6 patients who were diagnosed with parkinsonism before their deaths and who carried a pathogenic *LRRK2* p.G2019S mutation. The clinicopathological characteristics of these 6 individuals were heterogeneous (Table 2). Although all *LRRK2* patients presented with parkinsonism, only 3 progressed toward dementia (JHND964, JHND875, and JHND717). On histopathology, only 2 of the 6 *LRRK2* patients had typical, α-synuclein-positive Lewy body pathology consistent with a pathological diagnosis of Parkinson's disease (JHND823) or neocortical type LBD (JHND875; Fig. 3, panels D and E). One patient (JHND1206) showed nigral neuronal loss without any tau- or α-synuclein-positive inclusions (Fig. 3, panel F). The genetic profile of this particular patient was complex as she was also homozygous for the pathogenic p.N409S *GBA* mutation known to cause Gaucher disease. The 3 remaining cases showed neuropathological findings typical for PSP in the form of tau-positive neurons, neuropil threads, tufted astrocytes, and coil bodies without any accompanying Lewy body pathology (Fig. 3, panels A–C). Only 2 of these 3 patients fulfilled neuropathological criteria for a diagnosis of PSP, whereas the third case was interpreted as a *forme fruste* of PSP (JHND964). This observation provides strong support for the hypothesis that the *LRRK2* p.G2019S mutation can occasionally cause PSP, as previously suggested by rare incidental cases (Rajput et al., 2006; Ruffmann et al., 2012; Sanchez-Contreras et al., 2017).

For 3 of the 6 *LRRK2* p.G2019S mutation carriers (JHND875, JHND823, and JHND739), we had sufficient brain tissue available to perform a solubility analysis of α-synuclein (Supplementary Materials). Western blotting found modest amounts of urea-soluble α-synuclein only in 2 of our *LRRK2* p.G2019S cases that had Lewy body pathology on histopathological examination, whereas the third case, which was found to have tau-positive inclusions consistent with PSP on histopathological examination, had no α-synuclein deposition (JHND739) (Supplementary Materials). These data confirm that the patient diagnosed with PSP was void of insoluble, aggregated α-synuclein protein.

3.1.2. GBA *mutations*

Four individuals carried heterozygous *GBA* mutations, namely p.D448H (n = 2), p.T362I (n = 1), and p.R87Q (n = 1), whereas 1 patient (JHND1206) was homozygous for a p.N409S *GBA* mutation in addition to carrying an *LRRK2* p.G2019S mutation as described previously. All mutation carriers had parkinsonism on clinical presentation.

3.1.3. APP *mutations*

Five patients with high-level Alzheimer's disease pathology were found to have a highly penetrant missense mutation in *APP*, namely p.V717F (n = 3 patients) and p.V717L (n = 2). One of these 5 patients also had extensive Lewy body pathology (JHND924, p.V717F).

Furthermore, copy number variant analysis identified 3 siblings (JHND1189, JHND164, and JHND265) who suffered from early-onset Alzheimer's disease because of an *APP* gene duplication. Their clinical presentation was atypical for Alzheimer's disease, manifesting with prominent but heterogeneous psychiatric and behavioral symptoms in addition to progressive cognitive impairment (see detailed case descriptions in the Supplementary Materials). As a consequence, all 3 patients were clinically diagnosed with dementia with atypical features rather than Alzheimer's disease, and

Table 2
Genetic, clinical and pathological characteristics of patients with pathogenic mutations

Patient ID	Genetic characteristics			Clinical characteristics						Pathological features	
	Gene(s)	Mutation(s)	Genotype/ inheritance	Clinical diagnosis	AAO (decade of life)	AAD (decade of life)	Sex	Race	FH	Path. diagnosis	Comment
JHND964	*LRRK2*	p.G2019S	het (aut.dom.)	PDD	Fifth	Eighth	M	B	+	AD, nigral neuronal loss	Intermediate level AD pathology, no Lewy bodies, tau threads
JHND875[c]	*LRRK2*	p.G2019S	het (aut.dom.)	PDD	Seventh	Ninth	F	W	+	AD, LBD	Intermediate level AD pathology, LBD (neocortical subtype)
JHND739[c]	*LRRK2*	p.G2019S	het (aut.dom.)	PD	Eighth	Ninth	F	W	+	AD, PSP	High-level AD pathology, PSP, no Lewy bodies
JHND1206	*LRRK2*	p.G2019S	het (aut.dom.)	Parkinsonism	Fourth	Ninth	F	W	NA	AD, nigral neuronal loss	Intermediate level AD pathology, no Lewy bodies
	GBA	p.N409S	hom (aut.rec.)								
JHND717	*LRRK2*	p.G2019S	het (aut.dom.)	PDD	Fifth	Eighth	M	W	+	AD, FTLD-Tau	Intermediate level AD pathology, FTLD-tau (PSP subtype)
JHND823	*LRRK2*	p.G2019S	het (aut.dom.)	PD	Seventh	Eighth	M	W	NA	AD, PD	Low level AD pathology, PD (limbic type)
JHND800	*GBA*	p.D448H	het (aut.dom.)	PDD	Fifth	Seventh	M	W	-	AD, LBD	Low level AD pathology, LBD (neocortical type)
JHND1076	*GBA*	p.D448H	het (aut.dom.)	PDD	Seventh	Ninth	F	W	+	PD	LBD (limbic type), neurofibrillary tangles
JHND832	*GBA*	p.T362I	het (aut.dom.)	DLB	Fifth	Seventh	F	W	+	AD, LBD	Low level AD pathology, LBD (neocortical type)
JHND805	*GBA*	p.R87Q	het (aut.dom.)	PD/AD	Sixth	Seventh	M	W	+	AD, LBD	Intermediate level AD pathology, LBD (neocortical type)
JHND924	*APP*	p.V717F	het (aut.dom.)	AD	Fifth	Eighth	F	W	+	AD, LBD	High-level AD pathology, LBD (neocortical type)
JHND507[a]	*APP*	p.V717F	het (aut.dom.)	AD	Fifth	Sixth	M	W	+	AD	High-level AD pathology, no Lewy bodies
JHND360[a]	*APP*	p.V717F	het (aut.dom.)	AD	Fifth	Sixth	F	W	+	AD	High-level AD pathology, no Lewy bodies
JHND365[b]	*APP*	p.V717L	het (aut.dom.)	AD	Fifth	Sixth	F	W	+	AD	High-level AD pathology, no Lewy bodies
JHND204[b]	*APP*	p.V717L	het (aut.dom.)	AD	Seventh	Eighth	F	W	+	AD	High-level AD pathology, no Lewy bodies
JHND1189[d]	*APP*	Duplication	het (aut.dom.)	Dementia	Third	Sixth	M	W	+	AD, LBD, LD	High-level AD pathology, LBD (brainstem type), CAA
JHND164[d]	*APP*	Duplication	het (aut.dom.)	Dementia	Sixth	Sixth	M	W	+	AD	High-level AD pathology, CAA
JHND265[d]	*APP*	Duplication	het (aut.dom.)	Dementia	Sixth	Seventh	M	W	+	AD	High-level AD pathology, no Lewy bodies, CAA
JHND542	*PSEN1*	p.T116I	het (aut.dom.)	AD	Fourth	Fifth	M	W	(+)	AD	High-level AD pathology, no Lewy bodies
JHND170	*PSEN1*	pM139I	het (aut.dom.)	Dementia	Fourth	Fifth	M	W	-	AD	High-level AD pathology, no Lewy bodies
JHND132	*PSEN1*	p.I143T	het (aut.dom.)	AD	Fourth	Fifth	F	W	-	AD	High-level AD pathology, no Lewy bodies
JHND594	*PSEN1*	p.R269H	het (aut.dom.)	AD	Seventh	Eighth	M	W	-	AD	High-level AD pathology, no Lewy bodies
JHND623	*PSEN1*	p.A285V	het (aut.dom.)	AD	Fifth	Sixth	M	W	+	AD	High-level AD pathology, sparse Lewy bodies
JHND1007	*MAPT*	p.P301L	het (aut.dom.)	FTD	Sixth	Sixth	M	W	+	FTLD-Tau	Extensive phosphorylated tau deposition
JHND92	*MAPT*	p.R5L	het (aut.dom.)	AD	NA	NA	M	W	NA	AD	Low level AD pathology
JHND700	*GRN*	p.R110X	het (aut.dom.)	AD	Seventh	Ninth	F	W	NA	FTLD-TDP	Ubiquitin and TDP-43 positive inclusions
JHND650	*C9orf72*	Repeat exp.	het (aut.dom.)	ALS	Seventh	Seventh	M	B	+	ALS	Bunina bodies in anterior horn cells and pyramidal cells of Betz
JHND653	*C9orf72*	Repeat exp.	het (aut.dom.)	ALS	Fifth	Fifth	M	W	+	ALS	
JHND662	*C9orf72*	Repeat exp.	het (aut.dom.)	ALS	Sixth	Sixth	M	W	NA	ALS	
JHND669	*C9orf72*	Repeat exp.	het (aut.dom.)	ALS	Sixth	Sixth	F	W	NA	ALS	
JHND628	*C9orf72*	Repeat exp.	het (aut.dom.)	AD	Sixth	Seventh	M	W	NA	FTLD	Ubiquitin-positive inclusions
JHND676	*C9orf72*	Repeat exp.	het (aut.dom.)	ALS	Fifth	Sixth	F	W	+	ALS	Ubiquitin-positive inclusions
JHND644	*C9orf72*	Repeat exp.	het (aut.dom.)	ALS	Fifth	Fifth	M	W	+	ALS	
JHND625	*C9orf72*	Repeat exp.	het (aut.dom.)	AD	Seventh	Ninth	F	W	-	FTLD	Ubiquitin-positive inclusions, sparse neurofibrillary tangles
JHND1018	*C9orf72*	Repeat exp.	het (aut.dom.)	FTD	Seventh	Eighth	F	W	NA	FTLD	Ubiquitin and TPD-43 positive inclusions
JHND679	*SETX*	p.L389S	het (aut.dom.)	ALS	Second	Eighth	F	W	+	ALS	Anterior horn cell loss
JHND1207	*SETX*	p.L389S	het (aut.dom.)	ALS	First	Ninth	F	W	+	ALS	Anterior horn cell loss, ubiquitin-positive axonal swelling
JHND665	*SETX*	p.L389S	het (aut.dom.)	ALS	First	Seventh	M	W	+	ALS	Anterior horn cell loss
JHND1196	*SPAST*	p.R581X	het (aut.dom.)	HSP	Fourth	Ninth	F	W	(+)	Neurodegeneration	Frontoparietal atrophy, marked neurodegeneration
JHND1190	*CSF1R*	p.M766T	het (aut.dom.)	AD	Fourth	Fifth	F	W	NA	Neuroaxonal dystrophy	Neuroaxonal dystrophy
JHND1195	*CSF1R*	p.G589E	het (aut.dom.)	FTD	Sixth	Seventh	M	W	+	LD	Axonal spheroids and pigmented macrophages
JHND671	*CSF1R*	p.L868P	het (aut.dom.)	ALS	Sixth	Sixth	M	W	NA	ALS	Marked loss of motor neurons with reactive astrocytosis

Of note, age at onset and age at death are given as decades of life to protect privacy.
Key: AAO, age at onset; AAD, age at death; AD, Alzheimer's disease; aut.dom., autosomal dominant; aut.rec., autosomal recessive; B, black; exp., expansion; CAA, cerebral amyloid angiopathy; FTLD, frontotemporal lobar degeneration; HSP, hereditary spastic paraplegia; het, heterozygous; hom, homozygous; LBD, Lewy body dementia; LD, leukodystrophy; NA, data not available; NDD, neurodegenerative disease; PD, Parkinson's disease; PDD, Parkinson's disease dementia; W, white.
[a] JHND507 and JHND360 are first-degree relatives.
[b] JHND365 and JHND204 are first-degree relatives.
[c] JHND875 and JHND739 are third-degree relatives.
[d] JHND1189, JHND164 and JHND265 are first-degree relatives.

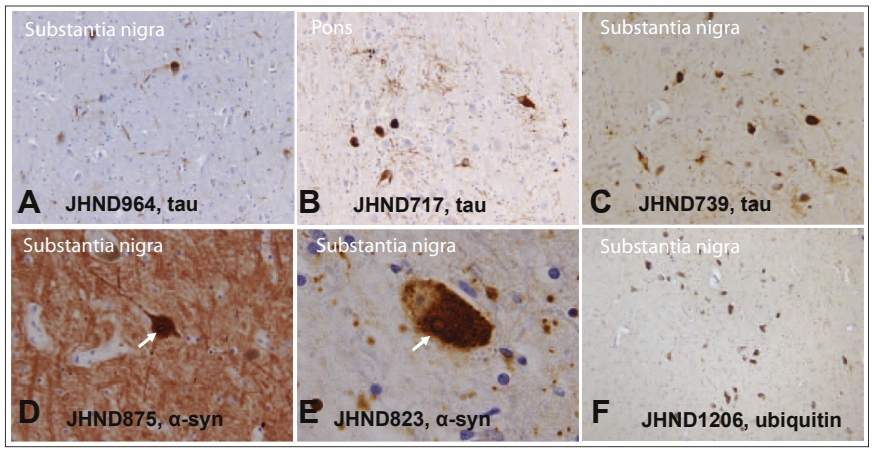

Fig. 3. The histopathological heterogeneity associated with *LRRK2*-related parkinsonism in 6 patients with a pathogenic p.G2019S *LRRK2* mutation. Three of 6 cases had tau-positive neurons, neuropil threads, tufted astrocytes, and coil bodies consistent with a diagnosis of PSP (panels A, B, C: 20× magnification), but no Lewy body pathology. Two patients were found to have nigral neuronal loss with associated Lewy body pathology (panels D, E; Lewy bodies indicated by arrow). One patient had nigral neuronal loss without tau- or α-synuclein-positive inclusion (panel F shows a few naturally pigmented nigral neurons; of note, this patient was also homozygous for the pathogenic p.N409S GBA mutation). Magnification is 40× for D, 100× for E, and 10× for panel F.

the correct diagnosis was only established on autopsy. This observation is consistent with clinical descriptions of other *APP* duplication patients that have reported atypical and heterogeneous dementia phenotypes, even within families (Guyant-Marechal et al., 2008; McNaughton et al., 2012).

In all 3 cases, the duplication encompassed a 3.5 Mb region on chromosome 21, including the entire *APP* gene. The pathological evaluation of these patients demonstrated high-level Alzheimer's disease pathology and cerebral amyloid angiopathy. However, 1 of the 3 siblings (JHND1189) also had Lewy bodies in his brainstem as well as leukodystrophy with relative sparing of subcortical U-fibers. Leukodystrophy has not been previously reported in patients with Alzheimer's disease related to duplications including the *APP* gene, and our observation broadens the pathological phenotype spectrum associated with this mutation. No other causative mutations in known leukodystrophy genes were observed in this patient.

3.1.4. PSEN1 *mutations*

Five cases with a pathological diagnosis of Alzheimer's disease were found to carry a pathogenic mutation in *PSEN1* (p.T116L, p.M139I, p.I143T, p.R269H, and p.A285B). While 4 of these patients presented with early-onset dementia, the fifth patient (carrying the p.R269H mutation) manifested with cognitive problems in his late 60s. Late-onset Alzheimer's disease has been previously reported for this particular mutation and, taken together, these observations suggest reduced penetrance of the p.R269H mutation compared with other *PSEN1* missense mutations (Larner et al., 2007). Aside from dementia, several atypical clinical features for Alzheimer's disease were noted in our *PSEN1* cases, including combativeness, dystonia, myoclonus, seizures, and gait apraxia that are similar to prior observations (Larner and Doran, 2006).

3.1.5. MAPT *mutations*

We identified 2 patients with pathogenic missense mutations in *MAPT*. One of these patients (JHND1007, p.P301L) presented clinically with behavioral variant FTD, and the patient's autopsy demonstrated marked accumulation of phosphorylated tau protein consistent with Pick's disease. This presentation is in line with prior observations of patients carrying this particular mutation (Hutton et al., 1998).

The second patient (JHND92) had low levels of Alzheimer's disease pathology and carried the p.R5L mutation in exon 1 of the *MAPT* gene. He had no PSP-like tau inclusions in his brain on autopsy. This particular missense mutation has once been previously reported as disease-causing in a single patient with PSP (Poorkaj et al., 2002). Another missense mutation at the same amino acid (p.R5H) has been reported in a small number of cases, including a Japanese sporadic FTD patient with PSP-like 4-repeat tau deposition on pathological evaluation, a pathologically confirmed Caucasian patient with apparently sporadic PSP, a small Japanese-American family with dementia and ALS without clear segregation, as well as 2 patients from a Taiwanese family with heterogeneous phenotypes (Hayashi et al., 2002; Leverenz et al., 2011; Lin et al., 2017; Poorkaj et al., 2002). None of these studies screened large control cohorts or demonstrated disease segregation, and it is questionable whether this variant is causally related to the described diseases. The p.R5H variant is present at low frequency in the East Asian population (allele frequency for R5H is 0.0064 according to Genome Aggregation Database). Based on these data, the p.R5H mutation should be classified as a variant of unknown significance. Furthermore, most known disease-causing *MAPT* mutations are within the microtubule binding domains (exons 9–13), and only a few disease-causing mutations have been shown to lie outside of these domains. Taken together, the pathogenicity of the p.R5H and p.R5L mutations is questionable.

3.1.6. GRN *mutation*

We identified 1 patient with FTD (JHND700) with a causative nonsense mutation in *GRN* (p.R110X). This patient was clinically diagnosed with Alzheimer's disease, although her pathology demonstrated severe atrophy of frontal, temporal, and parietal lobes. Immunohistochemistry identified TDP-43- and ubiquitin-positive inclusions consistent with a pathological diagnosis of frontotemporal lobar degeneration.

3.1.7. C9orf72 *repeat expansion*

Hexanucleotide repeat analysis of *C9orf72* identified 9 cases carrying a pathogenic expansion. Of these, 6 patients presented clinically with ALS, 1 case had a clinical presentation consistent with FTD, and 2 patients had cognitive changes that were clinically thought to be Alzheimer's disease, but neuropathological evaluations revealed the diagnosis of FTD. The median age at symptom onset was 52 years (range 44–69 years). Median survival in patients clinically presenting with ALS was only 3 years (range 1–4 years),

whereas patients clinically diagnosed with dementia displayed a longer median survival of 12 years (range 10–17 years).

3.1.8. SETX *mutations*

Three patients with familial ALS carried a pathogenic mutation in *SETX*. All 3 patients manifested disease at a young age with slowly progressive motor neuron disease and longer survival (Table 2). This presentation is consistent with prior reports of this rare form of motor neuron disease (Chance et al., 1998).

3.1.9. SPAST *mutation*

We identified an adult patient with a clinical diagnosis of hereditary spastic paraplegia, whose neurological symptoms started in the fourth decade of life. The pathological examination demonstrated marked neurodegeneration of her entorhinal cortex, pons, medulla, and spinal cord. Our molecular analysis confirmed the diagnosis by identifying a heterozygous nonsense mutation in *SPAST* (p.R581X). This particular mutation has been previously reported in an Italian patient with hereditary spastic paraplegia with a similar age at disease onset (Patrono et al., 2005).

3.1.10. CSF1R *mutations*

Mutations in *CSF1R* cause a rare, autosomal dominantly inherited neurodegenerative condition called adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (OMIM #221820). We were surprised to identify 2 cases with a pathogenic *CSF1R* mutation (p.G589E, and p.M755T) and 1 case with a likely pathogenic *CSF1R* mutation (p.L868P). This observation could indicate that this form of leukodystrophy is a more common form of neurodegeneration than previously recognized.

The clinical presentation of 2 of these patients (JHND1190 and JHND1195) was characterized by early-onset dementia with atypical features, including episodic confusion, seizures, and language impairment. The third case (JHND671) presented clinically with ALS, and this diagnosis was confirmed on autopsy. ALS has not been previously associated with pathogenic mutations in *CSF1R*. From this single observation, we cannot conclude that a causal relationship between this damaging mutation and ALS exists, but our data nominate *CSF1R* as a candidate gene that warrants additional exploration.

3.1.11. *Copy number variant analysis*

Aside from *APP* duplications described previously, copy number variant analysis identified 9 cases who carried *PARK2* copy number mutations, consisting of 6 heterozygous duplication carriers and 3 heterozygous deletion carriers. None of these *PARK2* copy number variant carriers were compound heterozygous with other pathogenic mutations in this recessively inherited gene, and these mutations were therefore unlikely to be disease-causing. Copy number variants were not identified in *SNCA* or *PSEN1*.

4. Discussion

Here, we describe a genetic evaluation of rare, pathogenic variants in a large cohort of pathologically confirmed neurodegenerative disease patients obtained from the Johns Hopkins Brain Resource Center. We identified 42 cases (approximately 3.4% of the total cohort) with damaging mutations in diverse neurodegenerative conditions, and we assessed the clinicopathological features of these individuals. This effort demonstrates the power of modern genomic technologies to rapidly screen important sample collections and to unravel the genetic architecture underlying diverse neurodegenerative diseases.

We made several crucial observations. First, our data strongly support the notion that the *LRRK2* p.G2019S mutation can present with clinically and pathologically heterogeneous syndromes, including Lewy body diseases, nonspecific nigral neuronal loss, and PSP. Limited studies have previously investigated the p.G2019S mutation in PSP cohorts (Gaig et al., 2008; Hernandez et al., 2005; Ruffmann et al., 2012; Sanchez-Contreras et al., 2017; Tan et al., 2006). A prior study identified a p.G2019S mutation in a single PSP case, but it remained unclear if this finding reflected the high prevalence of this mutation in the population or if it was truly disease-causing (Sanchez-Contreras et al., 2017). By contrast, our study strongly argues for a causal relationship between the p.G2019S mutation and PSP. Three of 6 *LRRK2* p.G2019S mutation carriers had pathological findings consistent with PSP pathological changes without any Lewy body pathology. Furthermore, the pathogenic p.R1441C, p.R1441H, p.T2310M, and p.A1413T *LRRK2* mutations have been previously reported in patients with PSP, providing further support for the notion that *LRRK2* mutations rarely cause PSP (Sanchez-Contreras et al., 2017; Spanaki et al., 2006; Trinh et al., 2015). This insight is important and implies that genetic testing for *LRRK2* mutations should be more broadly considered in parkinsonism patients with a family history of PSP or Parkinson's disease to establish a molecular diagnosis. These observations suggest that *LRRK2* mutations may have pleiotropic effects and raise the possibility that other genetic and environmental factors are involved in determining the exact phenotypic expressions of *LRRK2*-related neurodegeneration.

Second, our study highlights the utility of using high-throughput, low-cost genetic screening assays for molecular diagnostic purposes. This application is exemplified by the identification of disease-causing mutations in patients with early-onset dementia syndromes due to *CSF1R*, *PSEN1*, or *APP* mutations. Several of these patients were only correctly diagnosed on autopsy, or, in the case of *CSF1R* mutation carriers, the correct diagnosis was only revealed by our genetic analysis. Taken together, we argue that aside from the syndromic description of complex neurodegenerative phenotypes, a molecular genetic characterization of neurodegenerative syndromes should be attempted to correctly diagnose these patients, particularly because genetic screening is becoming more economical. We used the NeuroChip genotyping platform, which is rapid (3-day turn-around), affordable ($\sim$\$40/sample to screen several hundred thousand variants), and high-throughput (several thousand samples can be analyzed in a well-equipped laboratory each week).

Third, we made several unexpected observations. For example, 1 patient with a duplication encompassing *APP* also had leukodystrophy on pathological examination in addition to severe Alzheimer's disease pathology. We further identified a patient with ALS who carried a likely pathogenic *CSF1R* mutation. While these single observations do not allow us to draw definite conclusions, they could point toward novel, unrecognized disease associations that should be explored in future research studies.

Finally, another application of comprehensive genetic screening in a well-characterized patient series is that genomic knowledge can provide clarity about questionable pathogenicity labels. An example is the *MAPT* p.R5L mutation. This mutation has been nominated as disease-causing based on its location within a disease gene (Poorkaj et al., 2002). Another missense mutation at the same amino acid position, p.R5H, has been reported in a few clinically diagnosed neurodegenerative disease cases that lacked clear disease-segregation or screening in controls (Hayashi et al., 2002; Leverenz et al., 2011; Lin et al., 2017). The pathogenicity of mutations at this amino acid position therefore remains unclear. These mutations do not fulfill consensus criteria for pathogenicity based on current knowledge (Richards et al., 2015). The fact that we identified the p.R5L mutation in a case without any tau pathology also argues against a pathogenic role of this coding variant and

suggests that this mutation should be reclassified as a variant of unknown significance. This information is important for counseling of individuals carrying this variant, as genetic data do not conclusively demonstrate a pathogenic role of the p.R5L missense mutation.

5. Conclusion

Progress of molecular genomics technologies provides novel opportunities to gain critical insights into complex neurodegenerative syndromes. Although Mendelian forms of neurodegeneration only comprise a small proportion of the neurodegenerative disease population, defining molecular defects in these individuals reveals crucial phenotype correlations and clinical knowledge that allows for improved counseling of patients carrying pathogenic variants, and for studying the functional consequences leading to neurodegeneration.

Disclosure

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Authors' contributions: Study concept, design, and study supervision were done by SWS, JCT, and OP; patient recruitment was carried out by TD, AP, MSA, AEH, LSR, CCB, SMR, and LF; clinical phenotype data review was performed by SWS; sample preparations were carried out by JTG, CB, SWS, OP, and GR; review of pathological information was performed by OP and JCT; genotyping was carried out by JTG, NAM, and YA; variant validation was performed by JTG; bioinformatics was contributed by CB, JTG, FF, SA, RC, JD, MAN, and SWS; protein solubility analysis was performed by AM, MSS, and MRC; writing was contributed by SWS; critical revision of the article was carried out by all authors.

Appendix A. Supplementary data

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