Genetic and Pathological Links Between Parkinson's Disease and the Lysosomal Disorder Sanfilippo Syndrome

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ABSTRACT

Background: Parkinson's disease (PD) is a common neurodegenerative disorder of unknown etiology. The characteristic α-synuclein aggregation of PD is also a feature of Sanfilippo syndrome, a storage disorder caused by α-*N*-acetylglucosaminidase (*NAGLU*) gene mutations. We explored genetic links between these disorders and studied the pathology of Sanfilippo syndrome to investigate a common pathway toward α-synuclein aggregation.

Methods: We typed the 2 single-nucleotide polymorphisms that tag the common haplotypes of *NAGLU* in 926 PD patients and 2308 controls and also stained cortical tissue from 2 cases of Sanfilippo A syndrome using the anti-α-synuclein antibody, Per7.

Results: Allelic analysis showed an association between rs2071046 and risk for PD (P 1.3 × 10^{-3}). Intracellular α-synuclein accumulation was observed in the cortical tissue of both Sanfilippo A syndrome cases.

Conclusions: This study suggests a possible role of *NAGLU* in susceptibility to PD while extending evidence for α-synuclein aggregation in the brain in lysosomal storage disorders. Our findings support a mechanism involving lysosomal dysfunction more generally in the pathogenesis of PD. © 2011 *Movement* Disorder Society

Key Words: Parkinson's disease; alpha-synuclein; Sanfilippo syndrome; lysosomal disorders; genetics

Parkinson's disease (PD) is a common neurodegenerative disorder, characterized clinically by the triad of bradykinesia, rigidity, and tremor, alongside a wide range of nonmotor features.[1] The pathologic hallmark is loss of dopaminergic neurons from the substantia nigra pars compacta and the development of Lewy bodies containing α-synuclein.[2]

The etiology of PD is incompletely understood. Point mutations and multiplications in the α-synuclein gene (*SNCA*) are a cause of PD, implying a key role for this protein in the pathogenesis; however, collectively, monogenic forms of PD account for no more than 5% of cases.[3] Genome-wide approaches have confirmed common variations in tau and α-synuclein as important determinants of PD risk,[4,5] whilst the hypothesis-driven candidate gene approach continues to provide opportunities to evaluate pathogenic pathways involved in this complex disorder. By these means, a link has been established between PD and Gaucher disease, a glycolipid storage disorder caused by glucocerebrosidase (*GBA*) gene mutations, which are now recognized as the most common known genetic risk factors for parkinsonism.[6,7] The pathological phenotype of *GBA* mutation carriers comprises increased α-synuclein accumulation,[8] suggesting that aggregation of this protein might be linked to lysosomal dysfunction.

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This observation is supported by emerging reports of synucleinopathy across a range of storage disorders (reviewed in reference 9) including Sanfilippo syndrome (mucopolysaccharidosis type III MPSIII). MPSIII is an autosomal recessive neurodegenerative storage disease characterized by progressive motor and intellectual dysfunction with death in early adulthood. It is caused by deficiencies in 1 of 4 enzymes involved in the lysosomal degradation of the glycosaminoglycan heparan sulfate (HS), leading to accumulation of HS metabolites.[10] Each enzyme deficiency defines a different subtype of MPSIII—A, B, C, and D—and Hamano et al previously observed phosphorylated α-synuclein across various brain regions in 3 cases of MPSIIIB.[11]

We first sought to extend this previously reported pathological link between PD and MPSIII by investigating whether the risk for PD might be associated with a common variation in the α-*N*-acetylglucosaminidase gene (*NAGLU*; 17q21), heterogeneous mutations of which cause MPSIIIB. Second, we examined human cases of the related Sanfilippo A syndrome (MPSIIIA; caused by *N*-sulfoglucosamine sulfohydrolase gene mutations) to test the hypothesis that a more general impairment in the heparan sulfate clearance pathway could cause α-synuclein accumulation pathologically.

Patients and Methods

Genetic Analysis

DNA samples were obtained from 926 patients recruited from 2 centers in the United Kingdom: Cambridge (n = 711) and Newcastle (n = 215). All cases met UK PD Society Brain Bank criteria for idiopathic PD. A family history of PD was not an exclusion criterion because genetic risk factors for PD would be expected to be carried at a higher frequency in individuals with a family history. Sixty-two percent of patients were male, with a mean age at disease onset of 61.2 years (standard deviation, 12.2 years).

A total of 2308 controls included 141 unaffected spouses of PD patients from Cambridge, 152 unaffected spouses from Newcastle, and 2015 individuals from the British 1958 Birth Cohort. Forty-nine percent of controls were male.

Participants provided written informed consent and a blood sample from which DNA was extracted using conventional methods. Local ethical approval was granted by the Cambridge (UK) and Sunderland (UK) local Research Ethics Committees.

We analyzed 2 common single-nucleotide polymorphisms (SNPs)— rs2071046 and rs2676533—to tag the common *NAGLU* haplotypes (57% and 27% population frequencies) based on linkage disequilibrium (LD) and haplotype analysis of HapMap release 27 data using Haploview version 4.1. Genotyping was performed using Taqman Assays on a 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Allelic and genotypic analyses were performed using the Unphased package version 3.1.4.[12]

MPSIIIA Immunohistochemistry

Paraffin-embedded tissue sections from 2 human MPSIIIA cases were obtained from Great Ormond Street Hospital, London. The first case was a 15-year-old female who presented at age 6 with behavioral and language problems. The second was an 11-year-old male who presented at age 4 with developmental delay and hepatomegaly. Both cases had microencephaly and died of bronchopneumonia. Diagnoses were confirmed by enzymology. Control tissue (n = 1) was obtained from a 7-year-old female who died following an acute presentation of hypoglycemia with cerebral edema and herniation.

Immunohistochemistry was performed using the polyclonal anti-α-synuclein antibody, Per7 (1:1000), as previously described.[13] Sections were incubated with the primary antibody in TTBS with 1% goat serum overnight at 4°C. After incubation with biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA), staining was visualized using the ABC Elite Kit (Vector Laboratories, Burlingame, CA) and 3,3′-diaminobenzidine (Vector Laboratories, Burlingame, CA) as the chromogen. Immunostaining of the sections was analyzed using a Leitz DMRB microscope.

Results

Genotyping of *NAGLU* in PD

We genotyped 2 SNPs (rs2071046 and rs2676533) in 926 PD cases and 2308 controls to assess whether common variation in *NAGLU* is associated with PD risk. Overall genotyping success rates were >97%, and neither marker showed deviation from Hardy–Weinberg equilibrium. There were no inconsistencies amongst 391 subjects genotyped in duplicate. Allelic analysis showed that the rs2071046[C] allele was overrepresented in PD cases (77%) compared with controls (73%); see Table 1. Genotype analysis indicated that this association reflected an increased frequency of C homozygotes among cases, corresponding to an OR of 1.32 (95% CI, 1.13–1.55) for C homozygotes versus carriers of the G allele. We found no association between variation in rs2676533 and PD in this sample.

Immunohistochemistry of MSPIIIA Cases

Immunostaining of brain sections with the anti-α-synuclein antibody Per7 showed α-synuclein accumulation in the perikarya of cortical neurons in both cases of MPSIIIA (Fig. 1) but not in the control brains (data not shown).

Table 1. Allelic and genotype analyses for NAGLU

dbSNP	Allele	PD (freq)	Control (freq)	OR (95% CI)	χ^2	*P*
rs2071046	C	1351 (0.77)	3215 (0.73)	1.232 (1.084–1.401)	10.314	1.3×10^{-3}
	G	413 (0.23)	1211 (0.27)	Reference	Reference	Reference
rs2676533	A	1697 (0.93)	4210 (0.92)	1.206 (0.975–1.493)	3.036	0.081
	G	121 (0.07)	362 (0.08)	Reference	Reference	Reference
	Genotype					
rs2071046	C/C	524 (0.59)	1162 (0.53)	1.32 (1.13–1.55)	12.12	5×10^{-4}
	C/G	303 (0.34)	891 (0.40)	Reference	Reference	Reference
	G/G	55 (0.06)	160 (0.07)	Reference	Reference	Reference
rs2676533	A/A	791 (0.87)	1939 (0.85)	1.20 (0.96–1.50)	2.53	0.11
	A/G	115 (0.13)	332 (0.15)	Reference	Reference	Reference
	G/G	3 (0.00)	15 (0.01)	Reference	Reference	Reference

Allele/genotype counts and frequencies are given for PD cases and controls. Odds ratios, Chi-squared values and associated *P* values are given for a risk conferred by an allele/genotype versus reference.

Discussion

We have provided preliminary evidence for an association between PD and a common variant of the *NAGLU* gene, mutations of which are causative for MPSIIIB. More specifically, homozygosity for the more common C allele at SNP rs2071046 is increased in PD. The SNP of interest resides approximately 2.5 Mb upstream of the extended H1/H2 haplotype, which has been strongly associated with PD susceptibility and is not in linkage disequilibrium with SNPs previously implicated in the disease. We therefore suggest that variation in *NAGLU* itself may be independently associated with risk for PD.

The suggested relationship between PD and MPSIIIB bears comparison with the established relationship between PD and Gaucher disease,[7] implying a common underlying mechanism involving lysosomal dysfunction. The hypothesis that lysosomal dysfunction contributes to the shared α-synuclein pathology in these disorders is supported by our finding of α-synuclein accumulation in the cortical tissue of 2 postmortem cases of the related storage disorder MPSIIIA. To our knowledge, this is the first demonstration of α-synuclein aggregation in the brains of humans with MPSIIIA and extends previous findings in murine models.[14] Our pathological observation therefore adds to the repertoire of disorders featuring α-synuclein pathology and endorses the existence of converging pathways of neurodegeneration between synucleinopathies and lysosomal disorders more generally. It remains to be investigated whether *N*-sulfoglucosamine sulfohydrolase, the gene mutated in MPSIIIA, is also associated with risk for PD, and this should be the focus of future studies.

Proteolysis is performed by 2 major mechanisms both of which have been implicated in pathogenesis of PD: the ubiquitin-proteasome system and lysosomal-dependent autophagy.[15] Disruption of either pathway may reduce clearance and therefore cause accumulation of aggregate-prone proteins such as α-synuclein.[14] Indeed, disruption of autophagy with accumulation of α-synuclein has recently been proposed as the mechanism by which leucine-rich repeat kinase 2 (*LRRK2*) mutations, the most common cause of familial PD, may exert their effects.[16] The oligomerization and

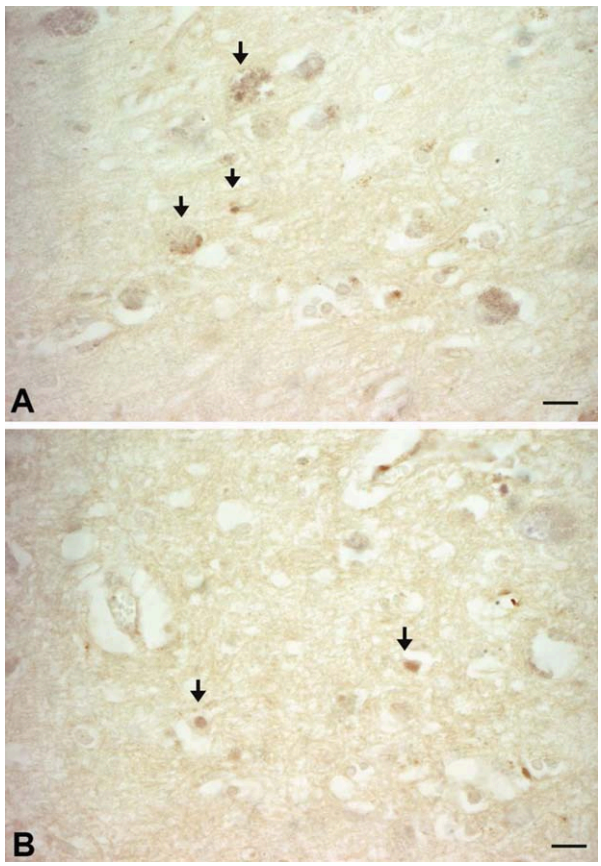

FIG. 1. α-Synuclein accumulation in the perikarya of cortical neurons in MPSIIIA stained with anti-α-synuclein antibody Per7 (arrows); n = 2. Bar = 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

accumulation of α-synuclein, a lipid-binding protein, may be facilitated by lipid dysregulation,[17] and lipids are known to accumulate as ubiquitous secondary storage products in lysosomal disease.[18] Indeed, altered fatty acid composition has been reported in PD brains.[19]

There are, however, other possible links between MPSIII and PD. For example, heparan sulfate (HS) modulates GDNF signaling, which in turn may be required for the survival and proliferation of populations of neurons including adult midbrain dopaminergic neurons. Exogenous HS (or, conceivably, excess HS, as may accumulate under mutant *NAGLU*) inhibits rather than enhances GDNF signaling[20] and by so doing could adversely affect the integrity of these populations of neurons in PD.

The limitations of association studies such as this include susceptibility to false-positive findings because of population stratification. We found no evidence, however, that factors such as region or source of recruitment confounded our observations. The high control:case ratio in this study compensated for the use of unselected control subjects (with a potential PD risk), which can theoretically cause the significance of findings to be underestimated. The SNP (rs2071046) of interest was not perfectly represented in the recent UK PD Genome Wide Association Study (GWAS), although we are encouraged by a comparable trend ($P = .01$) in the 2 proxy SNPs, rs7223784 ($r^2 = 0.881$) and rs647397 ($r^2 = 0.806$) with the alleles that are in LD with our risk allele, likewise overrepresented in PD.[4] Nonetheless, both our approach and the typical GWAS methods would not detect associations with rarer variants of the gene. The focus of future research, in line with *GBA* studies, should therefore be to investigate the prevalence of *NAGLU* mutations in PD as well as to study the rates of parkinsonism among obligate heterozygous relatives of MPSIII patients.

In conclusion, this study has generated preliminary evidence for a genetic association between MPSIIIB and PD. Taken together with our pathological findings of α-synuclein accumulation in MPSIIIA, this suggests that the pathway involved in HS degradation may play a role in α-synuclein accumulation in PD and supports the idea that lysosomal dysfunction contributes more ubiquitously to α-synucleinopathy and neurodegeneration. ■

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