Clinical/Scientific Notes

M.A. van Es, MD
P.W.J. van Vught, PhD
G. van Kempen
H.M. Blauw, MD
J.H. Veldink, MD, PhD*
L.H. van den Berg, MD, PhD*

DPP6 IS ASSOCIATED WITH SUSCEPTIBILITY TO PROGRESSIVE SPINAL MUSCULAR ATROPHY

Progressive spinal muscular atrophy (PMA) is a disorder characterized by loss of lower motor neurons resulting in progressive muscle weakness. It has been debated whether PMA is a distinct disease entity or should be considered a subtype of amyotrophic lateral sclerosis (ALS). PMA can progress to ALS and the disease course of PMA can be equally relentless, with death due to respiratory failure within 3 years.[1] Familiar patients with ALS with mutations in *SOD1* can lack UMN signs. Furthermore, pathologic studies of PMA have shown involvement of the corticospinal tract and ubiquinated inclusions, as also observed in ALS.

Sporadic ALS and PMA are complex diseases, with environmental and genetic factors contributing to disease susceptibility. Mutations in ALS cases have been found in *SOD1*, *ANG*, and *TDP-43*. However, in the majority of cases the genetic background of sporadic ALS is unknown. Over the last 2 years, several genome-wide association studies (GWAS) have been performed in ALS and have highlighted the discovery of three novel candidate genes, including *DPP6*.[2] This association has now been replicated twice by independent studies.[3,4]

Considering the clinical and pathologic overlap between ALS and PMA, we investigated the hypothesis that genetic variation in *DPP6* may be a risk factor for PMA.

Methods. A total of 155 patients with PMA and 806 healthy controls were included in this study. All participants provided informed consent and the study was approved by the local ethics committee. Baseline characteristics are provided in the (table).

Patients were diagnosed at the UMC Utrecht by experienced neurologists. All patients were above 18 years of age at onset and had no family history of motor neuron disease. All patients had clinical and electrophysiologic evidence of progressive LMN involvement (weakness, atrophy, and fasciculations) in more than one region (bulbar, cervical, thoracic, and lumbosacral) according to the 1998 revised El Escorial criteria.[5,6] Diagnosis was made after all other possible causes for symptoms had been excluded as described previously.[1] Details are provided in appendix e-1 on the *Neurology*® Web site at www.neurology.org.

Controls were individuals accompanying patients to our clinic and healthy individuals who enrolled in a prospective population-based study on ALS in The Netherlands (as described previously).[2] Details on genotyping procedures are provided in appendix e-1. Association analyses were performed using a χ^2 test on basic allele counts and under different models using the statistical analysis program PLINK.

Results. A χ^2 test on basic allele counts demonstrated a significant association between susceptibility to PMA and the C-allele of rs10260404 with $p = 0.03$ with odds ratio (OR) 1.30 (95% confidence interval [CI]: 1.02–1.60) (table). Further statistical analyses under different models revealed significant association for the genotypic ($p = 0.013$), recessive models (0.003), and the Cochran-Armitage trend test ($p = 0.032$). The highest disease risk was observed under a recessive model with an OR of 2.17 (95% CI: 1.42–3.31) for individuals with the CC genotype. Detailed results are shown in table e-1.

Discussion. A recent GWAS and subsequent independent studies have demonstrated that rs10260404 is a risk factor for ALS (pooled analysis $p = 9.89 \times 10^{-11}$ with an OR of 1.32).[2-4] Considering the clinical and pathologic overlap between PMA and ALS, we explored the possibility that the same genetic factors may influence susceptibility. We genotyped rs10260404 in 155 patients with PMA and 806 healthy controls. This revealed an association between the C-allele and susceptibility to PMA with a p value of 0.034. The observed minor allele frequency in the PMA group and the effect size are comparable to the findings in ALS. The identification of a common genetic risk factor for ALS and PMA, as well as the fact that 70% of patients with PMA develop ALS after 6 years and pathologic studies have shown corticospinal tract involvement in 68% of patients with PMA with ubiquinated inclusions, suggest that ALS and PMA may be strongly related or one disease. Our current data do not allow us to investigate the possibility that rs10260404

Supplemental data at www.neurology.org

Table Baseline characteristics of the studied populations and the results from association analyses for rs10260404

	No.	Male/female, %	Mean age, y	Allele frequency, % C	T	p Value	OR (95% CI)
PMA	155	69/31	58	43.4	56.6	0.034	1.30 (1.02–1.60)
Controls	806	58/42	59	37.0	63.0		
Previous studies in ALS							
Van Es et al.[2]							
ALS	1,767	58/42	59	41.6	58.4	5.04×10^{-8}	1.30 (1.18–1.43)
Controls	1,916	57/43	58	35.4	64.6		
Cronin et al.[3]							
ALS	221	54/46	61	41.6	58.4	0.029	1.36 (1.03–1.79)
Controls	211	53/47	58	34.4	64.6		
Del Bo et al.[4]							
ALS	266	65/35	61	42.9	57.1	6.60×10^{-3}	1.43 (1.11–1.85)
Controls	239	—	64	34.3	65.7		
Pooled analysis							
ALS	2,254	58/42	59	41.7	58.3	9.89×10^{-11}	1.32 (1.25–1.48)
Controls	2,366	57/43	58	35.2	64.8		

Mean age is given at onset for patients and at sampling for healthy controls. *p* Values were calculated using a χ^2 test on basic allele counts.
OR = odds ratio; CI = confidence interval; PMA = progressive spinal muscular atrophy; ALS = amyotrophic lateral sclerosis.

might be predictive for conversion of PMA to ALS, as all patients who did develop ALS after years of exclusively lower motor neuron dysfunction were excluded.

rs10260404 is located in intron 3 of dipeptidyl-peptidase-like protein 6, which is expressed predominantly in the nervous system. DPP6 affects the expression and gating of Kv4.2 channels, which play a role in the regulation of neurotransmitter release and neuronal excitability in the glutamatergic synapse.[7] This raises the intriguing possibility that DPP6 is involved in excitotoxicity, which is regarded as one the main processes in ALS pathogenesis. The replication of the association between *DPP6* and motor neuron disease makes it a novel molecular target for functional studies in these devastating diseases.

*These authors contributed equally.

From the Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, The Netherlands.

Supported by The Netherlands Organisation for Scientific Research (NWO), the Adessium Foundation, and the "Prinses Beatrix Fonds" (L.H.v.d.B.).

Disclosure: The authors report no disclosures.

Received October 21, 2008. Accepted in final form December 9, 2008.

Address correspondence and reprint requests to Dr. Leonard H. van den Berg, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, The Netherlands; l.h.vandenberg@umcutrecht.nl

ACKNOWLEDGMENT

The authors thank the patients and their families for participation.

1. Visser J, Van Den Berg-Vos RM, Franssen H, et al. Mimic syndromes in sporadic cases of progressive spinal muscular atrophy. Neurology 2002;58:1593–1596.
2. van Es MA, van Vught PW, Blauw HM, et al. Genetic variation in DPP6 is associated with susceptibility to amyotrophic lateral sclerosis. Nat Genet 2008;40:29–31.
3. Cronin S, Berger S, Ding J, et al. A genome-wide association study of sporadic ALS in a homogenous Irish population. Hum Mol Genet 2008;17:768–774.
4. Del Bo R, Ghezzi S, Corti S, et al. DPP6 gene variability confers increased risk of developing sporadic amyotrophic lateral sclerosis in Italian patients. J Neurol Neurosurg Psychiatry 2008;79:1085.
5. Brooks BR, Miller RG, Swash M, Munsat TL, for the World Federation of Neurology Research Group on Motor Neuron Diseases . El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis 2000;1:293–300.
6. de Carvalho M, Dengler R, Eisen A, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol 2008;119:497–503. Review.
7. Clark BD, Kwon E, Maffie J, et al. DPP6 localization in brain supports: function as a Kv4 channel associated protein. Front Mol Neurosci 2008;1:8.

B. Segarane, MSc*
A. Li, BSc*
R. Paudel, MSc
S. Scholz, MD
J. Neumann, MSc
A. Lees, MD
T. Revesz, MD
J. Hardy, PhD
C.J. Mathias, MD
N.W. Wood, MD
J. Holton, MD
H. Houlden, MD

GLUCOCEREBROSIDASE MUTATIONS IN 108 NEUROPATHOLOGICALLY CONFIRMED CASES OF MULTIPLE SYSTEM ATROPHY

Parkinson disease (PD), Lewy body dementia (LBD), and multiple system atrophy (MSA) are synucleinopathies whose primary pathogenic event is the deposition of inclusions composed of aberrantly fibrillized α-synuclein.[1] In PD and LBD, Lewy bodies are the key aggregate, whereas in MSA, α-synuclein accumulates in the form of oligodendroglial and neuronal cytoplasmic inclusions (GCIs and NCIs).[2,3]

Parkinsonian manifestations have been noted in a subset of patients with Gaucher disease and there is evidence that parkinsonism is more frequent among carrier relatives of patients with Gaucher disease.[4] In a remarkable study, the glucocerebrosidase (GBA) gene was sequenced in an American PD brain bank series where GBA mutations were detected at a much higher frequently than in controls (PD 21% vs control 4.5%).[5] These findings have since been replicated, mainly in Ashkenazi patient groups who have a higher mutation frequency but also in patients with clinically and pathologically diagnosed PD and LBD in a number of studies in different populations.[4] In a study of 75 neuropathologically confirmed synucleinopathies, GBA mutations were found in 23% of the cases with Lewy bodies.[6] The

frequency of GBA mutations around the world between 2.3 and 31% (depending on population) indicates that GBA mutations are one of the commonest genetic risk factors for PD.

GBA mutation carriers have a wide spectrum of phenotypes, ranging from classic L-dopa-responsive PD to LBD. In neuropathologic studies of PD/LBD cases, GBA mutations, α-synuclein inclusions, and Lewy bodies have been seen. This spectrum of clinical and pathologic features would suggest that MSA should also be a candidate to have GBA mutation.[3] Only 12 cases of MSA have been analyzed for GBA mutations and defects were seen in this handful of cases.[6]

We extracted DNA from the brain tissue of 108 neuropathologically confirmed British MSA cases that had been diagnosed according to brain bank criteria and 257 normal British controls. Mean age at onset was 58.2 ± 10.7 years (range 34–83), mean age at death 64.5 ± 10.2 years (39–87), mean disease duration 6.8 ± 2.9 years (2–16), and 48% were men. All exons and flanking intronic regions of the GBA gene were sequenced in MSA and control cases. To avoid amplifying and sequencing the GBA pseudogene we employed long range GBA PCR and then BigDye sequencing as previously described.[7]

In our MSA study group of 108 cases, we identified one heterozygous GBA mutation (c.904C>T; R262H), giving a mutation frequency of 0.92%. In the British controls, three heterozygous mutations (V497L, N409S, and R269Q) out of 257 cases were identified (1.17%). There was no significant difference between the two groups ($p = 0.66$). The single MSA case with the heterozygous R262H mutation was a woman with an age at onset of 44 years. She had parkinsonian, cerebellar, and autonomic features (MSA–mixed type) with no family history. She died at age 51 years and the neuropathology revealed widespread GCIs and NCIs with a predominance in striatonigral structures. There were no Lewy bodies.

One limitation of our study is the small sample size. Our study has a power of 80% to detect variants with an OR >1.61 or <0.63 at a significance level of 0.05. The results of this study indicate that GBA mutations are not common etiologic players in Caucasian patients with MSA. We cannot exclude that GBA mutations confer modest or low risk to disease. Furthermore, we did not sequence risk variants in regulatory regions (such as the promotor region or untranslated regions). Mutations in these regions would therefore have been missed.

The unexpected role of GBA mutations has been demonstrated in several populations and is undoubtedly a highly significant risk factor for PD and LBD. More importantly, GBA mutations reveal a direct link between the lysosomal protein pathway and the clearance or the development of α-synuclein aggregates into Lewy bodies. Our study indicates that GBA mutations are not associated with MSA in the population that we analyzed, and that this branch of the ceramide pathway is unlikely to be associated with all types of primary α-synuclein deposition.

*These authors contributed equally.

From the Department of Molecular Neuroscience and Reta Lila Weston Laboratories (B.S., A.L., R.P., S.S., J.N., A.L., T.R., J. Hardy, N.W.W., J. Holton, H.H.), Institute of Neurology, University College London, UK; Molecular Genetics Section (S.S.), Laboratory of Neurogenetics, National Institute on Aging and Health, Bethesda, MD; and Neurovascular Medicine Unit (C.J.M.), Faculty of Medicine, Imperial College London at St. Mary's Hospital, London, UK.

Supported by the Medical Research Council (MRC), The Michael J. Fox Foundation, The Sarah Matheson Trust, The Brain Research Trust (BRT), The BMA (Vera Down grant), the Reta Lila Weston Institute for Neurological Studies, and the Intramural Research Program of the NIH, National Institute on Aging, project number: Z01-A400057-05. This work was undertaken at University College London Hospitals/University College London, which received a proportion of funding from the Department of Health's National Institute for Health Research Biomedical Research Centers funding scheme.

Disclosure: The authors report no disclosures.

Received September 19, 2008. Accepted in final form December 11, 2008.

Address correspondence and reprint requests to Dr. Henry Houlden, Department of Molecular Neuroscience and Reta Lila Weston Laboratories, Institute of Neurology, University College London, Queen Square, UK WC1N 3BG; h.houlden@ion.ucl.ac.uk

ACKNOWLEDGMENT

The authors thank the patients with MSA and their families, the Queen Square Brain Bank, and the Parkinson's Disease Society Brain Bank for tissue and their help with this study.

1. Wenning GK, Jellinger KA. The role of alpha-synuclein in the pathogenesis of multiple system atrophy. Acta Neuropathol 2005;109:129–140.
2. Dickson DW, Liu W, Hardy J, et al. Widespread alterations of alpha-synuclein in multiple system atrophy. Am J Pathol 1999;155:1241–1251.
3. Ozawa T, Paviour D, Quinn NP, et al. The spectrum of pathological involvement of the striatonigral and olivopontocerebellar systems in multiple system atrophy: clinicopathological correlations. Brain 2004;127:2657–2671.
4. Sidransky E. Heterozygosity for a Mendelian disorder as a risk factor for complex disease. Clin Genet 2006;70:275–282.
5. Lwin A, Orvisky E, Goker-Alpan O, LaMarca ME, Sidransky E. Glucocerebrosidase mutations in subjects with parkinsonism. Mol Genet Metab 2004;81:70–73.
6. Goker-Alpan O, Giasson BI, Eblan MJ, et al. Glucocerebrosidase mutations are an important risk factor for Lewy body disorders. Neurology 2006;67:908–910.
7. Stone DL, Tayebi N, Orvisky E, Stubblefield B, Madike V, Sidransky E. Glucocerebrosidase gene mutations in patients with type 2 Gaucher disease. Hum Mutat 2000;15:181–188.