ORIGINALARTICLE

Neurofilament M gene in a French-Canadian Population with Parkinson's Disease

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ABSTRACT: ***Background:*** Recently, a single base pair substitution (G1747A) mutation of the neurofilament M (NF-M) gene was reported in a French-Canadian patient with early onset Parkinson's disease (PD). Three unaffected siblings were found to be heterozygotes for the NF-M Gly336Ser mutation but, to date, no other affected PD individuals have been found with a similar mutation. No other individuals with Parkinson's disease and of similar ethnic background have been screened for this mutation. ***Methods:*** We screened 102 French-Canadian patients with definite PD and 45 French-Canadian controls for this substitution in the *NF-M* gene using a PCR-restriction enzyme digestion method. ***Results:*** None of the patients or controls carried this mutation. ***Conclusion:*** Our results would indicate that this mutation is not common even in a PD population of similar ethnic background and suggest this change represents a rare variant. However, these results do not exclude the possibility that other mutations in this gene could be present.

RÉSUMÉ: Le gène du neurofilament M chez des patients canadiens-français atteints de la maladie de Parkinson. ***Introduction:*** Une mutation impliquant une substitution d'une seule paire de bases (G1747A) dans le gène du neurofilament M (NF-M) a été rapportée récemment chez un patient Canadien-français atteint de la maladie de Parkinson (MP). Trois membres de sa fratrie qui ne sont pas atteints de la maladie sont hétérozygotes pour la mutation NF-M Gly336Ser, mais jusqu'à maintenant on n'a trouvé une telle mutation chez aucun autre individu atteint de la MP. ***Méthodes:*** Nous avons recherché cette substitution dans le gène NF-M au moyen de la méthode par technique PCR et digestion enzymatique par une enzyme de restriction chez 102 patients canadiens-français atteints de MP certaine et 45 témoins canadiens-français. ***Résultats:*** Aucun des patients ou des témoins n'était porteur de cette mutation. ***Conclusion:*** Selon nos résultats, cette mutation n'est pas fréquente, même chez des patients atteints de la MP et ayant la même origine ethnique. Il s'agit donc d'une variante rare. Cependant la présence d'autres mutations dans ce gène n'est pas exclue.

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Parkinson's disease (PD; MIM 168600) is a common progressive neurologic disorder with a prevalence of ~125 per 100,000 Canadians.[1] Clinically, it is characterized by: resting tremor, rigidity, bradykinesia and postural instability.[2] Until recently, the etiology of the majority of cases of PD was unknown. Even though environmental and occupational exposures may play a role in the disease process,[3] the importance of the genetic factors has been highlighted by the identification of mutations in six different genes.[4-7]

Recently, a single base pair substitution (G1747A) mutation of the neurofilament M (*NF-M*) gene (GenBank: accession number, Y00067) was identified in a 23-year-old French-Canadian patient with early onset PD. This mutation resulted in a Gly336Ser amino acid substitution. Her symptoms began at the age of 16 with resting tremor, bradykinesia, rigidity and normal cognitive function. She developed severe motor fluctuations within three years of treatment that required bilateral deep brain stimulation with excellent response. The patient's mother had similar symptoms and signs that onset in her teens, responded initially to carbidopa/levodopa, but then developed increasing bradykinesia and dementia and died after a 15-year course. The maternal grandmother and eight siblings were studied with three siblings in their early 30s and 40s found to be heterozygotes for the NF-M Gly336Ser mutation.[8] This suggests that this mutation could be autosomal dominant with a reduced penetrance. This

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mutation was not identified in 342 controls of which 113 were of French-Canadian heritage. Forty-eight other non-French-Canadian PD families were also screened for mutations in the *NF-M* gene but no other mutations were identified. As no other French-Canadian PD individuals have been screened for this mutation it is unknown how commonly the NF-M Gly336Ser mutation could be occurring in this population. In this study we explore whether this mutation could play a role in a large cohort of PD individuals of French-Canadian heritage.

METHODS

All subjects gave informed consent for the protocol that had been approved by the local institutions'Ethics Committee. Blood samples were collected from two clinics (one in Ottawa, Ontario, one in Montreal, Quebec, Canada) from 2001-2003 as part of an ongoing effort to explore the role of genetics in PD in a Canadian cohort focusing on individuals of French-Canadian descent. All participants completed a structured questionnaire, underwent a history and examination by a neurologist with extensive experience in PD (M.P. or D.G.). Diagnosis of PD and exclusionary criteria suggestive of another disease were based on previously published criteria.[9]

Genomic DNA was extracted from above blood samples using the standard protocols of the QIAGEN kit (QIAGEN Inc. Mississauga, Canada). Oligonucleotide primers covering the NF-M G1747A gene mutation were designed according to the reported primer sequence: NF-M16 (sense:5'-GACCTTCTGG-CCCAGATCCA-3') and NF-M15 (antisense:5'-TGGCCGAG-GCCGCGGTTCCTA-3'). Polymerase chain reaction (PCR) conditions were carried out with a thermal cycler setting of 94ºC for 2 min, then 34 cycles at 94ºC for 45 s; 59ºC for 45 s; 72ºC for 45 s; and a final extension at 72ºC for 10 min. Polymerase chain reaction products were then digested with the restriction enzyme FspI (New England Biolab, Beverly, MA) according to the manufacture's instructions and electrophoresed on a 1.5% agarose gel. The G1747Amutation creates a FspI restriction site. After PCR and FspI digestion, an individual with the wild type alleles would show a single band at 373 bp, conversely the G1747A allele/mutation would result in two extra bands at 274 bp and 99 bp.

To confirm the activity of the enzyme FspI, a 2961 bp-pBluescript II KS (+) with 2 FspI cutting sites was tested. The conditions for digestion reaction were 1 µl of pBluescript II KS (+) DNA(300 ng/µl), 2 µl of buffer (Newbuffer 4), 0.5 µl of FspI and 16.5 µl of dd H_2O for two hours at 37ºC. After digestion, the pBluescript II KS (+) reaction with FspI enzyme shows two bands at 1789 bp and 1172 bp.

RESULTS

Among the 102 French-Canadian patients with the clinical diagnosis of PD, 89 of them had both parents of French-Canadian origin while the other 13 patients had only one parent of French-Canadian origin; 65 patients were male while 37 patients were female; 31 patients had an age of onset less than or equal to 49 years, while 71 patients had an age of onset greater than or equal to 50 years. Twenty-four PD individuals had a positive family history of more than one relative with PD while nine patients had more than two relatives with PD.

Restriction digestion of the PCR products of the *NF-M* gene did not show the NF-M Gly336Ser mutation in any of the 102 patients or in the 45 controls. This result indicates that the Gly336Ser mutation is not common even in a French-Canadian PD population.

DISCUSSION

Neurofilament M is one of the three neurofilament subunits-the light, medium, and heavy neurofilaments (NF-L, NF-M and NF-H) which are the most abundant intermediate filaments in neurons, accounting for more than 85% of the total protein content.[10] Intermediate filaments are a family of 10-nM diameter structures that interact with actin microfilaments and microtubules to form the cytoskeletal scaffolding in eukaryotic cells. In many neurons, once stable synapses have formed, neurofilaments accumulate robustly as axonal diameter increases. Although the neurofilaments are not essential for the survival of neurons, alterations in their expression or stoichiometry at least in some animal models, suggests that abnormalities in these proteins can cause neurologic dysfunction.[11,12] Among the three neurofilament proteins, NF-L seems to play the most important role to form the filament backbone while NF-M and NF-H form the filament side arms to associate to other filaments or microtubules. Because of the phosphorylation sites in the C-terminus domains of NF-M and NF-H , the orientation of NF side arm projections and the NF spacing could be regulated by their phosphorylations. As for the relationship between the NF and neurological diseases in humans, both an A998C transversion which converts a conserved Gln333 amino acid to proline and a C64T transition, which converts a pro22 amino acid to serine in the NF-L gene were found to be associated to Charcot-Marie-Tooth type 2 (CMT2) disease.[13,14] Both insertions and deletions in the NF-H side-arm are found to be the risk factors for amyotrophic lateral sclerosis.[15,16] The Gly336Ser mutation in the NF-M occurs within the Arg-Gly-Thr-Lys-Glu sequence thought to be the important domain for NF-M to interact with other proteins.

Growing evidence indicates that abnormalities in NF-M could lead to the development of PD. Neurofilaments are major components of Lewy bodies found in PD and dementia with Lewy bodies.[17] Mice carrying a human mutant superoxide dismutase transgene have been found to overexpress neurofilament and display Lewy body like inclusions that are related to neuronal cell death.[18] The chromosome 8p region that contains the *NF-M* gene has been identified as a possible susceptibility locus for familial PD in one genome screen.[19] In addition, the more direct implication by finding a Gly336Ser sequence variant in at least one person with PD. This mutation in the *NF-M* gene of a French-Canadian PD patient implies that a structural disruption of the NF-M could lead to the abnormal assembly of neurofilaments and finally to the degeneration of dopaminergic neurons of the substantia nigra.[8] No other PD patients have been found to have similar mutations suggesting this mutation could be a rare variant versus a truly pathologic mutation.[8,20] However, no other PD individuals of similar ethnic background had been screened for this mutation. In our large series of PD individuals of French-Canadian descent (of which 25% had a positive family history) we could not identify anyone with a similar mutation. Possible reasons that none of our PD

patients nor the controls had this mutation include: this mutation arose from a more recent event (for example, in one of the patient's great grandparents) and therefore is not related to a founder population in the French-Canadian population; that mutations in other exons or splice sites of the *NF-M* gene could be present which were not screened for in our cohort; the reported patient with NF-M Gly336Ser mutation may not have had typical Parkinson's disease but a different neurodegenerative disease with parkinsonism as a feature. If she had a juvenile onset form of PD (onset age 16), it would be unusual for her to have such rapid progression of disease that required bilateral deep brain stimulation after three years. Even though her mother did have a treatment responsive juvenile onset parkinsonism, she also appeared to have a more malignant course and developed a dementia that would be unusual for most young onset cases of PD.

After screening for this mutation in French-Canadian PD patients and controls, our results suggest that this mutation does not play a major role for the development of PD in individuals of similar ethic background. Functional characterization of the Gly336Ser substitution in the *NF-M* gene is needed to assess whether it is truly pathologic or just a rare sequence variant.

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