

# Analysis of *LRRK2* functional domains in nondominant Parkinson disease

**Abstract**—A comprehensive sequence analysis of 29 exons that code for the functional domains of *LRRK2* in 160 nondominant Parkinson disease (PD) patients was performed. Novel variant screening in a further 470 sporadic PD patients and 630 controls revealed two novel variants (R1067Q and IVS33 + 6 T>A), which are likely to be pathogenic in five patients. One patient presented initially with a typical essential tremor phenotype, expanding the phenotypic spectrum of *LRRK2* mutations.

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Linkage studies have identified at least six genes that result in neurodegeneration associated with Parkinson disease (PD).<sup>1</sup> The most recently identified of these is *LRRK2* (PARK8; OMIM #607060), which encodes a putative protein kinase (leucine-rich repeat kinase 2). Mutated *LRRK2* results in clinical and pathologic features indistinguishable from typical PD. At least seven different mutations have been identified thus far that occur within evolutionary conserved regions thought to be functionally important.<sup>2–4</sup> These regions include a leucine-rich repeat (LRR), a Roc (Ras in complex protein) domain, a C-terminal of Roc (COR) domain, a kinase (MAP-KKK) domain, and a WD40 domain.<sup>2</sup> They occupy the C-terminal half of the peptide and are encoded by exons 23–51 of the gene.

Patients with PD from various white populations harbor mutations in *LRRK2* and ages of disease onset vary from the third to the seventh decade of life.<sup>2–6</sup> Original linkage to the PARK8 locus was established in a Japanese family,<sup>7</sup> although the contribution of *LRRK2* to PD in Asian populations has not yet been assessed.

Originally associated with a dominant mode of inheritance, variable penetrance has been noted. It is therefore important that screening efforts include sporadic patients, as well as those where inheritance patterns are unclear. Two recent studies analyzed the G2019S mutation in sporadic PD and found this particular mutant frequency to be around 1%.<sup>4,6</sup> We report a comprehensive analysis of all the exons coding for the putative functional domains in a large cohort of patients with sporadic PD.

**Methods.** A total of 630 consecutive PD patients (group A: age at onset [AOO] <55 years or positive family history; group B: AOO ≥55 years and without family history) were initially recruited from the movement disorder clinics of two major tertiary institutions in Singapore (Singapore General Hospital and National Neuroscience Institute). PD diagnosis was made in accordance with the UK Parkinson Disease Society Brain Bank Clinical Diagnostic Criteria of PD by two movement disorder neurologists. All PD subjects were previously screened negative for the *LRRK2* G2019S mutation (appendix E-1 on the *Neurology* Web site at [www.neurology.org](http://www.neurology.org)). The mean AOO in group A was 46.5 ± 10.9 years (range 25 to 88 years). In all, 75% of patients were sporadic and 25% had a positive family history but were not compatible with a dominant mode of inheritance (appendix E-2). About 58% of patients were men. Chinese ethnicity accounted for 88%, 7% were Malay, and 5% were Indian. Comprehensive sequence analysis of the 29 exons was carried out in 160 patients in group A.

The control group comprised 630 individuals without neurodegenerative disease, with similar sex and ethnicity proportions as the patient group. All study subjects were examined by the authors. Institutional ethics committees approved this study and informed consent was obtained from all participants.

After we had determined putative *LRRK2* pathogenic variants in group A, we further screened for these specific variants in the 470 sporadic PD patients (group B) with a mean AOO of 64.8 ± 8.4 years (range 55 to 88 years). The frequency of these mutations was determined in both patients and controls.

**Sequence analysis.** A total of 29 exons (23 to 51) of *LRRK2* (based on NCBI accession number AY792511) were examined by direct sequencing, as previously described.<sup>2</sup> For details of genotyping and in-silico analysis, please refer to appendix E-3 and table E-1.

**Results.** **Sequence analysis.** Sequence analysis in group A revealed two potentially pathogenic variants. The first was a heterozygous G>A transition at bp 3200, resulting in a predicted arginine-to-glutamine substitution at amino acid 1067 (R1067Q). This residue is within the LRR, and arginine at this position is conserved in vertebrates (figure). The second was a heterozygous T>A transversion 6bp downstream of exon 33 (IVS33 + 6 T>A), which

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## Leucine-Rich Repeat Kinase 2

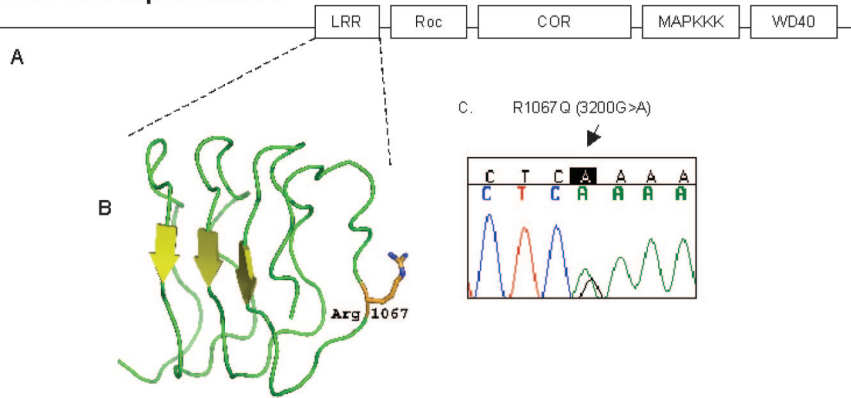


Figure. (A) *LRRK2* protein and position of the putative functional domains. (B) Predicted topology of the LRR, showing position of R1067. (C) Sequence chromatogram showing a heterozygous R1067Q variant (3200G>A).

changes the consensus donor splice site sequence AGAGTRAGT (IVS33 + 6 T>A is shown in bold (figure E-1). In-silico analysis predicts this will decrease the information content (Ri) from 6.1 to 4.5, resulting in a threefold decrease in spliceosome binding capability.

**Screening.** Screening in group B and the control group identified no more occurrences of R1067Q. However, we found three more patients heterozygous for IVS33 + 6 T>A, but this variant was not present in controls.

Case descriptions of *LRRK2* positive patients are shown in (appendix E-4).

**Discussion.** This comprehensive sequence analysis of 29 exons that code for the functional domains of *LRRK2* in 160 nondominant PD patients and novel variant screening in a further 470 sporadic PD patients and 630 controls identified two novel variants that may be pathogenic.

The R1067Q variant lies within the LRR domain (see figure). The primary function of LRRs appears to involve protein-protein interactions.<sup>8</sup> Our protein modeling predicts that the hydrophilic arginine residue at position 1067 is exposed to the outside. Therefore, the glutamine mutant could allow new protein-protein/protein-ligand interactions to be facilitated without altering the structure of the domain. This is consistent with the pathogenic gain-of-function mechanism proposed for other *LRRK2* mutations.<sup>9</sup> LRRs are implicated in cellular localization signaling; mutations within LRRs of the major histocompatibility complex II activator (CIITA) result in altered nucleocytoplasmic transport in vivo.<sup>10</sup> It remains to be seen whether mutations in the LRR of *LRRK2* have a similar effect. The phenotype of the patient carrying R1067Q is similar to typical PD and resembles *LRRK2* positive patients.

Zimprich et al. reported synonymous exonic variation 6 bp from the exon/intron boundary of exon 24, segregating with disease in a dominant family.<sup>2</sup> Splice-site sequence dictates the strength of the spliceosome-splice junction interaction and thus splice-site use. The consequences of donor splice-site mutations may involve the skipping of one or more exons or intron retention, depending on the strength of adjacent donor/acceptor signals. Our novel IVS33 + 6 T>A (Ri = 4.5) is predicted to possess

30% efficiency as a donor splice signal, as variants retaining a Ri value  $\geq 2.4$  do not usually abolish splicing completely. The IVS33 + 6 A variant could therefore result in a reduction of normal *LRRK2* levels, either with or without the presence of abnormal species. Of interest is that one patient presented with a typical essential tremor (ET) phenotype and her symptoms responded well to propranolol. Rest tremor and the full Parkinsonian features were only noticed 8 years later. Although the etiologic link between ET and PD is under debate, the lack of family history and excellent response to propranolol do suggest that this phenotype spectrum may be secondary to the consequence of the *LRRK2* variant. Presence of pleomorphic pathology in postmortem patients alludes to the possibility that *LRRK2* may be implicated in various neurodegenerative processes.<sup>2</sup>

Because all of our five positive *LRRK2* variant patients were nonfamilial, we could not provide proof of cosegregation with disease phenotype. However, the two novel variants were not detected in >1200 normal chromosomes and we have provided in-silico evidence of the potential functional impact of these two variants.

Our results suggest that *LRRK2* mutations may be responsible for a small percentage of nondominant PD cases (<1%) in Asia. Interestingly, our sequence analysis did not reveal the presence of any previously reported mutations seen in whites. Hence the spectrum of mutation in *LRRK2* may be population-specific and warrants further investigation.

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## Migrating intraventricular cysticercus during MRI

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A 10-year-old boy presented with headache and vomiting of 1-week duration. MRI revealed marked hydrocephalus. T1-weighted images showed a cystic lesion isointense to CSF, with a thin wall and a small nodule (scolex) in the left temporal horn (figure, A and B). Contrast images performed 20 minutes later revealed migration of the cyst to the occipital horn (see the figure, C and D). Migration of intraventricular cysticercosis is rarely reported.<sup>1</sup> Demonstration of scolex on MR is pathognomonic of neurocysticercosis.<sup>2</sup> The cyst, which was seen changing its position during the course of MRI, is rather unusual.

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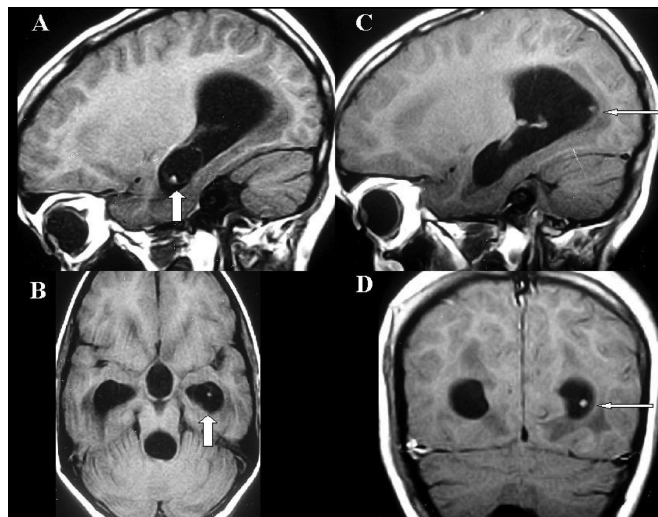


Figure. (A, B) T1-weighted spin echo images in sagittal and axial planes show hydrocephalus and a cyst with scolex in the temporal horn of left lateral ventricle (arrow). (C, D) T1-weighted spin echo postcontrast images in sagittal and coronal planes done 20 minutes later demonstrate the gravity-dependent migration of the cyst to the occipital horn (arrow).

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