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Comparative Genomic Hybridization Solves a 14-Year-Old *PARKIN* Mystery

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In 2001, we and our colleagues[1] reported on two families with *PARKIN* mutations, one of which (family Ph) presented with only one *PARKIN* mutation. Mutations in the *PARKIN* (*PARK2*) gene cause early-onset autosomal-recessive parkinsonism. At that time, we concluded: "…a hemizygous mutation may confer increased susceptibility to typical Parkinson's disease", and further speculated: "…either the hemizygous deletion makes these individuals more susceptible to parkinsonism/dystonia or there are multiple other, as yet undetected parkin mutations… ."[1] Two follow-up publications[2,3] came to similar conclusions. Here, we present new data using microarray comparative genomic hybridization (array CGH) to reanalyze the *PARKIN* gene in family Ph, which resolve these questions.

In our original publication in *Annals of Neurology*, semiquantitative polymerase chain reaction (PCR) was performed for assessment of *PARKIN* copy number variants (CNVs). This method was introduced by Lücking et al. in a seminal publication for *PARKIN* gene characterization.[4] Under the premise that we might have missed the second mutation in the *PARKIN* gene, we recently reanalyzed all available DNA samples of family Ph for CNVs using array CGH, a method now commonly utilized in clinical settings for the detection of submicroscopic genomic rearrangements. The experiments were performed on a 1M Agilent CGH array (Agilent Technologies, Santa Clara, CA). Data were processed at Population Diagnostics, Inc. (Oxford, UK). Patient genomic DNA was prepared from brain (Ph1 and Ph15) and whole blood (Ph6 and Ph13).

We detected a second mutation in the *PARKIN* gene (Figure 1). The unaffected mother (Ph6) is a carrier of a 118-kb duplication including exon 6 of the *PARKIN* gene, which was not previously identified with semiquantitative PCR. Thus, the early onset of symptoms in the three affected brothers

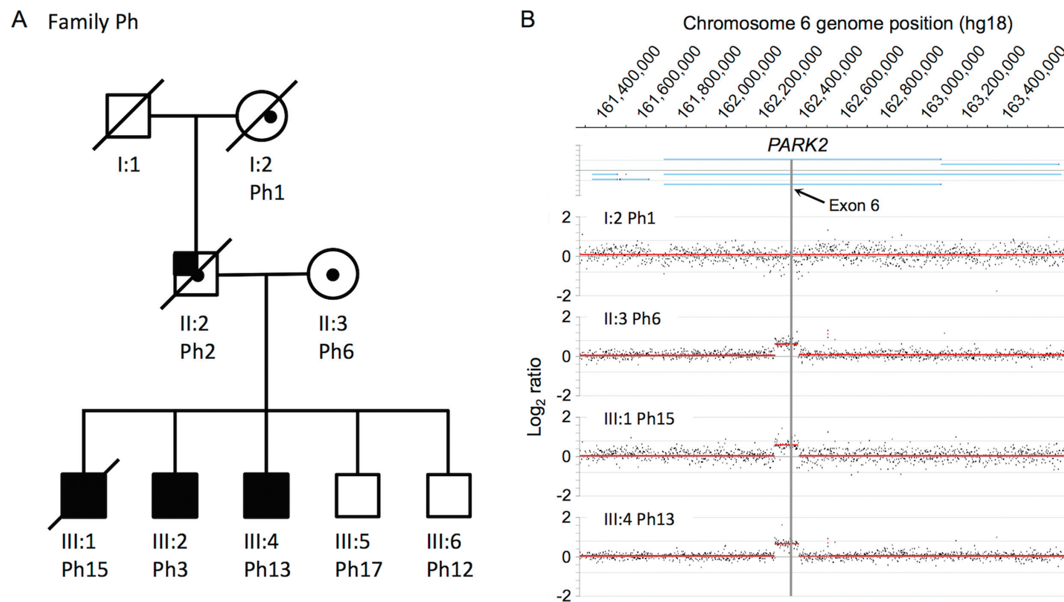

FIGURE 1: (A) Pedigree of family Ph. Identifiers with Roman numerals are from Tan et al;[3] Ph identifiers are based on Farrer et al.[1] For clarity, we omitted additional family members from the pedigree that were not analyzed in this study. I:2 and II:2 were carriers of the originally reported *PARKIN* c.337_376del40 mutation. Brain autopsies from I:2 and II:2 were normal for age and had no neuropathological findings related to Parkinson's disease (PD). Note: II:2 was diagnosed with PD, but proved to have essential tremor. II:3 is unaffected and a carrier of the newly identified *PARKIN* 118Kb exon 6 duplication. III:1 and III:4 carry the *PARKIN* 118Kb exon 6 duplication in addition to the *PARKIN* c.337_376del40 mutation. Detailed clinical case descriptions are published in Tan et al.[3] (B) Array CGH results. Depicted is the 6q26 chromosomal region that includes the *PARKIN* gene. Genome coordinates (NCBI36/hg18) are displayed at the top (x-axis). The gene annotation track is followed by the CGH profile for 4 Ph family members wherein the y-axis corresponds to the Log2 ratio value of the patient DNA relative to a sex-matched reference DNA. A value of 0 corresponds to no change in copy number relative to a reference genome, whereas duplicated chromosomal regions have Log2 ratio values of ∼0.6 (copy number changes are demarcated by red line segments). The location of exon 6 within the duplication is indicated (vertical black line), and the hg18 genome coordinates are chr6:162,243,072-162,360,682.

(Ph15 [age at onset = AO 44[3]], Ph3 [AO24], and Ph13 [AO24]) and the clinical presentation with parkinsonism/dystonia is consistent with compound heterozygous mutations, *PARKIN* [c.337_376del40]; [118Kb exon 6 duplication], as commonly described for autosomal-recessive *PARKIN* parkinsonism.

It has been reported, in several studies, that the semiquantitative multiplex PCR we used in our original report is biased in favor of detection of deletions over duplications, which suggests that heterozygous duplications (change from two to three copies) are underestimated and missed when semiquantitative PCR is used.[5] In conclusion, for accurate copy-number analysis, which is critical for clinical genetic testing and counseling, we recommend the use of array CGH when genomic rearrangements are suspected in genetic forms of Parkinson's disease.

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Authorship

B.S. and J.W.L. designed the study. E. H. and P.S.E. analyzed array CGH data. All authors wrote and edited the manuscript.

Potential Conflicts of Interest

E.H. and P.S.E. are employees of Population Diagnostics.

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