α-Synuclein Locus Triplication Causes Parkinson's Disease

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Mutations in the α-synuclein gene (*SNCA*) in the Contursi kindred (*1*) implicated this gene in Parkinson's disease (PD). Subsequently, α-synuclein was identified as the major component of Lewy bodies, the pathological hallmark of PD, and of glial cell cytoplasmic inclusions (*2*).

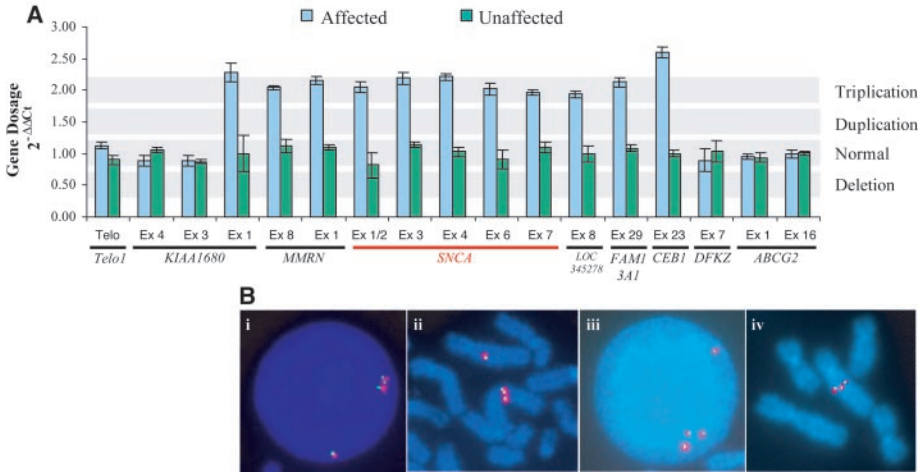

Fig. 1. (**A**) Gene dosage analysis of *SNCA* and flanking genes from affected family member 9-77. Results are the mean of six replicates and expressed as $2^{-\Delta\Delta Ct} \pm$ SD. (**B**) FISH analysis of interphase (i and iii) and metaphase (ii and iv) chromosomes from EBV-transformed lymphocytes of patient 9-77 showing *SNCA* triplication. Red, labeled PAC 27M7 marker spanning *SNCA*; green, 11-kb fragment corresponding to the promoter region and first 3 exons of *SNCA*. Tight apposition of the two metaphase chromatids precludes resolution of double minutes.

We examined a large family with autosomal dominant PD (average age of onset, 34 years), ranging clinically from dementia with Lewy bodies to typical PD (*3*). Neuropathological examination of affected members revealed profound pathology including extensive Lewy bodies and some glial cell cytoplasmic inclusions. Screening this family for mutations in, or linkage to, *SNCA* was negative. Linkage analysis revealed a chromosome 4p15 haplotype segregating with parkinsonism and essential tremor, with suggestive evidence for linkage to *PARK4* [multipoint logarithm of odds (LOD) = 2.64 at *D4S1609*] (*4*). However, an unaffected individual who did not share the 4p15 haplotype became ill. This prompted a second genome-wide search at higher resolution, which revealed a haplotype co-segregating with disease over 26 cM (*D4S2367–D4S1560*), with a multipoint LOD of 3.50 at *D4S2460*. The *SNCA* genotypes were inconsistent with previous data, leading to initial exclusion; re-evaluation of the original linkage revealed a sample swap. Resequencing of *SNCA* failed to reveal pathogenic mutations.

The heterozygous single nucleotide polymorphisms in the *SNCA* promoter and in intron 5 suggested that deletion of this region was unlikely. Reverse transcriptase–polymerase chain reaction (RT-PCR) amplification of *SNCA* revealed only transcripts of normal length and sequence in affected family members. Analysis of intragenic markers *MG4S2* and *MG4S5* at the *SNCA* locus showed apparent examples of non-mendelian inheritance, which could be interpreted as multiple alleles. Quantitative real-time PCR amplification of *SNCA* exons yielded results consistent with whole gene triplication (Fig. 1). To confirm *SNCA* triplication, we performed fluorescent in situ hybridization (FISH) of chromosomes from Epstein-Barr virus (EBV)-immortalized lymphocytes from an affected family member (9-77). *SNCA* triplication in this family segregates with parkinsonism but not postural tremor (fig. S1).

Using quantitative PCR of flanking annotated genes in the human sequence, we determined the extent of triplication. The telomeric end of the triplication occurs within the model gene *KIAA1680*, between exons 1 and 3, a distance of 181 kb; the centromeric end occurs between exon 23 of the cyclin E binding protein gene (*CEB1*) and exon 7 of hypothetical protein *DKFZp761G058*, an interval of 243 kb (fig. S2). The triplicated region is between 1.61 Mb and 2.04 Mb in size and contains 17 annotated or putative genes (table S1). This region includes *SNCA*, at least 1.2 Mb flanking the centromeric sequence, and more than 280 kb flanking the telomeric sequence. Carriers of the *SNCA* triplication are predicted to have four fully functional copies of *SNCA*, with doubling of the effective load of an estimated 17 genes. It is possible that one or more of these genes rather than *SNCA* is responsible for disease pathology or that structural mutation in a gene at the end of the triplication is the pathogenic event. Parsimony argues, however, that increased dosage of *SNCA* is the cause of PD in this family.

These results are consistent with haplotype data suggesting that genetic variability in the *SNCA* promoter contributes to the risk of developing PD (*5*). They support evidence that mutant α-synuclein behaves differently from the wild-type protein in a quantitative rather than qualitative manner. Finally, the disease process in this family may resemble the etiology of Alzheimer's disease in Down syndrome, where overexpression of the *APP* gene due to chromosome 21 trisomy is the key event.

References and Notes

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Materials and Methods
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