

# The $\alpha$ -Synuclein Gene and Parkinson Disease in a Chinese Population

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**Objective:** To study the Ala53Thr and Ala30Pro mutations of the  $\alpha$ -synuclein gene in a large number of Chinese patients with Parkinson disease (PD) as well as controls.

**Methods:** We recruited 183 Chinese patients with sporadic PD, 17 with younger-onset PD (onset age <50 years), and 7 with PD and a positive family history as well as 227 unaffected Chinese control subjects from the outpatient departments of 2 major hospitals in Hong Kong. All subjects were assessed for the diagnosis of PD by a consultant neurologist or geriatrician. Subjects were interviewed with a standard questionnaire that also questioned for family history. Venous blood samples were obtained from the subjects and genomic DNA was extracted and studied for the presence of Ala53Thr mutation in exon

4 and Ala30Pro mutation in exon 3 of the  $\alpha$ -synuclein gene using a polymerase chain reaction restriction fragment length polymorphism method.

**Results:** None of the Chinese PD patients or controls had either the Ala53Thr (exon 4) or Ala30Pro (exon 3) mutation of the  $\alpha$ -synuclein gene.

**Conclusion:** We failed to discover Ala53Thr or Ala30Pro mutations in a large number of Chinese patients with PD and control subjects, adding to the emerging consensus that variations in the  $\alpha$ -synuclein gene are associated with PD in few families worldwide.

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**T**HE GENE that codes for the presynaptic protein  $\alpha$ -synuclein is associated with dominantly inherited parkinsonism in the Italian Contursi kindred and 3 unrelated Greek families.<sup>1</sup> In these families, affected members have a missense mutation G209A in exon 4, which results in an exchange of an alanine to a threonine at position 53 of the amino acid sequence (Ala53Thr). A second  $\alpha$ -synuclein mutation associated with Parkinson disease (PD) has been recently described by Kruger et al.<sup>2</sup> This variant is characterized by a cytosine-to-guanine transversion at nucleotide position 88 of the coding sequence (exon 3), which leads to a change at position 30 of the amino acid sequence (Ala30Pro).  $\alpha$ -Synuclein is a precursor to constituents of Alzheimer disease plaques and is found in Lewy bodies, the eosinophilic inclusion bodies that define neuropathological characteristics of PD. The function of  $\alpha$ -synuclein is unknown, although it is thought to be involved with neuronal plasticity and may mediate binding between structural pro-

teins.<sup>3</sup> Recent studies have failed to find the Ala53Thr mutation in subjects of European Caucasian ancestry with familial,<sup>4</sup> younger-onset,<sup>5</sup> and sporadic<sup>6</sup> PD. As for the Chinese, there has been only one small study<sup>7</sup> looking for the Ala53Thr mutation in 65 cases of sporadic PD in Taiwan Chinese patients. We therefore undertook a large-scale study to look for both Ala53Thr and Ala30Pro mutations. We investigated 183 sporadic, 17 younger-onset (onset age of younger than 50 years), and 7 Chinese patients with a positive family history together with 227 unaffected Chinese subjects for the presence of the Ala53Thr and Ala30Pro mutations in the  $\alpha$ -synuclein gene.

## RESULTS

The patients with PD and a positive family history consisted of 2 female siblings, 2 with an affected mother, 1 with an affected father, brother, and uncle, and 2 with secondary relatives with PD. Two control subjects reported secondary relatives with PD. None of the Chinese patients with PD or controls possessed ei-

## SUBJECTS AND METHODS

Patients and controls were recruited from the outpatient departments of the United Christian and Prince of Wales hospitals in Hong Kong. All subjects were assessed by a consultant neurologist or geriatrician for the diagnosis of PD. The criteria for diagnosis is in accordance with those described by Maranganore et al.<sup>8</sup> In the selection of controls, 10-year age group, sex, and locality of residence were taken into consideration to make the control group as comparable to the PD group as possible. (For more details of the study, refer to Chan et al.<sup>9</sup>)

The subjects were interviewed with a standard questionnaire and were questioned for family history of PD. If there was a family history, further details regarding which relatives were affected (ie, parents, siblings, uncles, aunts, nephews, or nieces) were obtained. Venous blood samples were taken from the subjects, and genomic DNA was extracted by the salting out method.<sup>10</sup>

The patients and controls were screened for the presence of the Ala53Thr mutation in exon 4 of the  $\alpha$ -synuclein gene (the Contursi mutation) using the polymerase chain reaction (PCR) *Ysp45I* restriction fragment length polymorphism (RFLP) method described by Polymeropoulos et al.<sup>1</sup> DNA that was obtained from an affected Contursi kindred member and contained the Ala53Thr mutation was kindly donated by Polymeropoulos et al and used as a positive control. The primer sequences used for the DNA amplification were 5'-GCTAATCAGCAATTTAAGGCTAG-3' (forward) and 5'-GATATGTTCTTAGATGCTCAG-3' (reverse). The amplification reaction was performed in a 50- $\mu$ L volume that contained 100 ng of template DNA, 300 ng of each primer, 2-mmol/L magnesium chloride, 0.24-mmol/L

deoxynucleotidetriphosphates, and 2 U of DNA polymerase (Red Hot DNA polymerase; Advanced Biotechnologies, Surrey, England). The samples were subjected to an initial denaturation at 94°C for 5 minutes followed by 17 cycles of denaturation at 94°C for 15 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds. The second phase of the amplification reaction involved 18 cycles of denaturation (94°C for 15 seconds), annealing (50°C for 30 seconds), and extension (72°C for 1 minute 30 seconds). The 216-base pair (bp) PCR product was digested with the restriction enzyme *Tsp45I* (New England Bio Labs, Beverly, Mass) according to the supplier's protocol, resolved on a 3% agarose gel (agarose 1000; Life Technologies, Melbourne, Australia), and visualized using ethidium bromide staining. Mutant alleles yield restriction fragments of 128 and 88 bp using this technique (**Figure**).

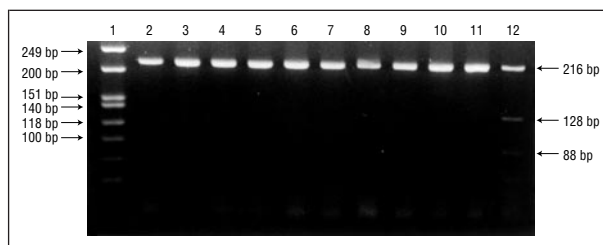
The Ala30Pro mutation in exon 3 of the  $\alpha$ -synuclein gene was examined using the *MvaI*-based PCR-RFLP method previously described by Kruger et al.<sup>2</sup> In this case, the forward and reverse primers were 5'-AAGTGTATTTATGTTTTCC-3' and 5'-AACTGACATTTGGGGTTTACC-3', respectively. The PCR reactions (25  $\mu$ L) contained 100 ng of template DNA, 20 pmol/L of each primer, 2.1-mmol/L magnesium chloride, 0.225-mmol/L deoxynucleotidetriphosphates, and 1 U of DNA polymerase. The samples were subjected to an initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 54°C for 1 minute, and extension at 72°C for 1 minute. A final extension period of 72°C for 5 minutes completed the reaction. The 192-bp PCR products were digested with *MvaI* restriction enzyme and resolved using 8% polyacrylamide gel electrophoresis followed by silver staining. Alleles containing the Ala30Pro mutation yield 136- and 56-bp fragments following *MvaI* digestion.

ther the Ala53Thr (exon 4) or the Ala30Pro (exon 3) mutation of the  $\alpha$ -synuclein gene.

The Figure shows that DNA from an affected Contursi kindred subject who possesses the Ala53Thr mutation yields RFLPs characteristic of the mutant allele. No such fragments were found for any of the Chinese patients and controls.

### COMMENT

The failure to discover the Ala53Thr or Ala30Pro mutations in a large number of Chinese patients with PD and control subjects adds to the emerging consensus that variations in the  $\alpha$ -synuclein gene are associated with PD in few families worldwide. Moreover, studies have thus far failed to reveal other mutations in the coding region of the  $\alpha$ -synuclein gene in large groups of patients with familial<sup>11,12</sup> and sporadic<sup>13</sup> PD. Studies are yet to determine whether any variations in the regulatory or intronic regions of the  $\alpha$ -synuclein gene are associated with PD. Efforts may be better spent in elucidating the mechanism by which the Ala53Thr and Ala30Pro variants of  $\alpha$ -synuclein lead to neurodegeneration in individuals possessing these mutations. Such mechanistic information may suggest other candidate



Restriction fragment length polymorphism assay for the Ala53Thr mutation in exon 4 of the  $\alpha$ -synuclein gene. All DNA samples from 207 Chinese patients with PD and 227 unaffected controls yielded a single DNA band, which corresponded to the undigested 216-base pair (bp) polymerase chain reaction product (lanes 2-11 display representative samples). DNA from an affected member of the Contursi kindred (lane 12) yielded 128- and 88-bp fragments together with the 216-bp fragment, indicating the presence of the Ala53Thr mutation. Lane 1 contains the  $\phi$ X174/*HinI* DNA marker.

genes with causative variants potentially more common in patients with PD worldwide than the Ala53Thr or Ala30Pro variants of  $\alpha$ -synuclein.

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