α-Synuclein promoter Rsal T-to-C polymorphism and the risk of Parkinson’s disease

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Summary. Increased α-synuclein expression may be involved in the pathogenesis of Parkinson’s disease (PD). We investigated the association of Rep1 microsatellite and Rsal T-to-C substitution in the α-synuclein promoter region with the risk of PD by a case-control study. The Rsal C/C genotype and C allele were found less frequently in PD patients than in controls. A reduced risk of the Rep1-Rsal 0-C haplotype (OR = 0.57, 95% CI = 0.36–0.90) with PD was evident. The quantitative real-time PCR study showed that the α-synuclein mRNA expression was increased (although not significantly) in PD patients with Rsal T/T genotype or Rep1-Rsal 0-T haplotype as compared to T/C genotype or 0-C haplotype. Reporter constructs containing the Rsal C allele drove significantly lower transcriptional activity compared with the Rsal T allele in both IMR32 and 293 cells. The findings suggest that the Rsal T-to-C substitution may have a functional relevance to the susceptibility to PD.

Keywords: Parkinson’s disease, α-synuclein, Rep1 dinucleotide repeat, Rsal T-to-C substitution, polymorphism and disease association.

Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disease characterized by resting tremor, rigidity, and bradykinesia. The characteristic pathological features of PD include juxtanuclear ubiquitinated proteinaceous inclusions in neuronal perikarya and neuronal processes (Lang and Lozano, 1998). An interaction between environmental factors and genetic predisposition, most of which is not yet known, is thought to cause PD. Causal mutations in the gene for alpha-synuclein (α-synuclein), parkin, ubiquitin carboxy-terminal hydrolase, DJ-1, PINK1, and LRRK2 have been identified (Paisan-Ruiz et al., 2004; Vila and Przedborski, 2004). However, mutations in these genes do not explain the occurrence of disease in most sporadic patients.

α-Synuclein, the main component of Lewy bodies (Spillantini et al., 1997), is
a presynaptic protein encoded by the \( \alpha \)-synuclein gene on chromosome 4q21-23 (Polymeropoulos et al., 1996). Three mutations, A53T, A30P and E46K, were identified (Polymeropoulos et al., 1997; Kruger et al., 1998; Zarranz et al., 2004) in the highly conserved N-terminal portion of the \( \alpha \)-synuclein protein. In the transgenic and cellular studies, it was shown that the mutant A53T and A30P \( \alpha \)-synuclein proteins were more prone to fibrillogenesis, suggesting that \( \alpha \)-synuclein may have an important role in the development of PD (Narhi et al., 1999). Recently, a triplication of the \( \alpha \)-synuclein gene was found to cause PD in two distinct families (Singleton et al., 2003; Farrer et al., 2004). Triplication resulted in a doubling of mRNA in brain tissue and of protein in blood (Miller et al., 2004), indicating that a mere overexpression of wild-type \( \alpha \)-synuclein is sufficient to cause the disease. Duplication of the \( \alpha \)-synuclein gene also caused a late age-of-onset and slowly progressive clinical phenotype (Chartier-Harlin et al., 2004; Ibanez et al., 2004). In addition, a polymorphic mixed dinucleotide repeat (referred to as Rep1 microsatellite) has been reported in the \( \alpha \)-synuclein promoter (Xia et al., 1996) with various expression levels among different alleles (Chiba-Falek and Nussbaum, 2001). An association between the Rep1 with PD has been shown (Kruger et al., 1999; Tan et al., 2000; Farrer et al., 2001; Pals et al., 2004), although this has been disputed (Parsian et al., 1998; Izumi et al., 2001; Khan et al., 2001; Tan et al., 2003).

The aim of this study was to test whether the genetic variability in the \( \alpha \)-synuclein gene might be involved in the susceptibility of Taiwanese to PD. We sequenced and genotyped the Rep1 microsatellite and a downstream RsaI T-to-C substitution. The \( \alpha \)-synuclein expression level was quantified to correlate with genetic variability. The potential association of the RsaI T-to-C substitution was also examined in a functional study.

**Materials and methods**

**Subjects**

The study sample included 252 patients (50.0% women), aged 37–91 years (mean, 67.6 years), with probable PD (Calne et al., 1992), and enrolled from the neurology clinic of Chang-Gung Memorial Hospital. All patients exhibited at least two of the four cardinal signs of PD: resting tremor, cogwheel rigidity, bradykinesia, or postural-reflex impairment. Exclusion criteria included a prior history of multiple cerebrovascular events or other causes of parkinsonian symptoms (e.g. brain injury or tumor, encephalitis, antipsychotic medication). The 257 controls (48.3% women), aged 23–98 years (mean, 60.1 years), were composed of unrelated healthy adult volunteers. All the subjects gave consent for the studies.

**Analysis of Rep1 microsatellite and RsaI T-to-C polymorphisms**

DNA was extracted from leukocytes, and the Rep1 dinucleotide repeat was determined by PCR amplification (Kruger et al., 1999) and resolved in a linear polyacrylamide gel on an automated MegaBACE Analyzer. Seven alleles, allele −2, −1, 0, 1, 2, 3, and 4 (255–267 bp) (Xia et al., 1996), were identified. The results were verified by cloning all 7 different alleles into pGEM-T Easy (Promega) and sequenced. To determine the mixed sequence repeat, 44 individuals homozygous for the common allele 0, 1, or 2 were sequenced directly. While no single nucleotide polymorphism (SNP) rs2619367 (T-to-G variation 79 bp downstream of the repeat) was observed, a T-to-C substitution 111 bp downstream was detected. The T-to-C substitution can be examined by RsaI restriction assay (referred to as RsaI T-to-C polymorphism). For the Rep1 allele 0, 227 and 32-bp fragments appeared on the variant C allele, whereas the 259 bp fragment was found on the common T allele. Genotype and allele frequencies for Rep1 and RsaI T-to-C sites were calculated, and differences between patients and controls were tested by the \( \chi^2 \) test of association. The pair-wise haplotype frequencies were computed and chi-square tested for association. Odds ratios with 95% confidence intervals (95% CI) were calculated to test for association.

**Analysis of mRNA expression**

Total RNA was extracted from lymphocytes using the PAXgene Blood RNA Kit (QIAGEN). The RNA was DNase treated, quantitated, and reverse transcribed to cDNA using Superscript III (Invitrogen). The \( \alpha \)-synuclein-specific primers (forward, 5'-TCTGCGCT TTCCACCCTCGT-3'; reverse, 5'-CTGTCGTCGAA