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Brief communication

SNCA mutation p.Ala53Glu is derived from a common founder in the Finnish population

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ABSTRACT

Mutations in *SNCA* are rare causes of familial Parkinson's disease (PD). We have previously described a novel p.Ala53Glu mutation in 2 Finnish families. To assess this mutation's frequency among Finnish PD patients, we screened 110 PD patients (mean age-of-onset 60 years) from Western Finland by Sanger sequencing of the third coding exon of *SNCA*. In addition, a sample of 47 PD subjects (mean age-of-onset 53 years) originating from Southern and Eastern Finland were studied using next-generation sequencing covering *SNCA*. Only one new individual with the p.Ala53Glu mutation was identified, confirming that this mutation is a rare cause of PD in the Finnish population. To search for a possible common origin of the p.Ala53Glu mutation, haplotype analysis was conducted in 2 families and in a patient from a third family (6 affected subjects) using both STR markers and a genome-wide SNP array. The results show that patients with the p.Ala53Glu mutation share a haplotype spanning a minimum of 5.7 Mb suggesting a common founder.

1. Introduction

The *SNCA* gene on 4q22.1 codes for a 17-kDa protein predominantly expressed in brain, especially in presynaptic terminals. The exact functions of SNCA are still somewhat unclear, but it is likely involved in modulation of synaptic activity by participating in vesicle release (Bendor et al., 2013). Aggregated SNCA proteins form inclusions that are the classical findings in neurodegenerative synucleinopathies: Parkinson's disease (PD), dementia with Lewy bodies and multiple system atrophy (MSA). Multiplications (Chartier-Harlin et al., 2004; Singleton et al., 2003) and point mutations, p.Ala30Pro (Krüger et al., 1998), p.Glu46Lys (Zarranz et al., 2004), p.His50Gln (Appel-Cresswell et al., 2013), p.Gly51Asp (Kiely et al., 2013), p.Ala53Thr (Polymeropoulos et al., 1997), p.Ala53Glu (Pasanen et al., 2014), of *SNCA* have been implicated in rare, autosomal dominant PD spectrum disorders.

We previously reported a novel *SNCA* p.Ala53Glu mutation in a Finnish family with atypical Parkinson's disease in 3 patients. Neuropathological examination of the index patient showed highly abundant alpha-synuclein pathology throughout the brain and spinal cord with features of both MSA and PD. The *SNCA* p.Ala53Glu mutation was seen in all the 3 affected family members

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(Pasanen et al., 2014). Functional studies have shown that the p.Ala53Glu mutation reduces alpha-synuclein fibril formation and enhances toxicity in cells under stress due to mitochondrial impairment (Ghosh et al., 2014; Rutherford and Giasson, 2015). The p.Ala53Glu mutant protein also has a lower membrane binding affinity than the wild type protein (Ghosh et al., 2014).

Since the first report, the p.Ala53Glu mutation was reported in another Finnish family with autosomal dominant PD by Martikainen et al. (2015). The mutation was detected in 2 affected patients of the family.

These findings prompted us to investigate how common this mutation is among Finnish PD patients. We screened a larger cohort of PD patients for the p.Ala53Glu mutation. In addition, we performed a haplotype analysis that demonstrated a shared haplotype in all individuals with the mutation. Our findings show that the *SNCA* p.Ala53Glu mutation is rare and originates from a common founder in the Finnish population.

2. Material and methods

2.1. PD patients

2.1.1. Families F1 and F2

Families F1 and F2 have been described before (Martikainen et al., 2015; Pasanen et al., 2014). Seven DNA samples were available from these families.

2.1.2. PD patient cohorts

Autopsy-derived deep-frozen brain samples were available from 45 PD patients, who were neuropathologically verified as PD (24 males, 21 females; mean age at onset: 64.2 years [range 47–80, SD ± 7.7 years]; mean disease duration: 11.5 years [range 3–20, SD ± 4.4 years]; mean age at death: 75.7 years [range 50–88, SD ± 7.3 years]). Three patients also had neuritic plaques suggestive of Alzheimer's disease. Blood-derived DNA samples were available from additional 65 patients with clinically diagnosed PD (40 males, 25 females, mean age at onset: 57.8 years [range 37–79, SD ± 9 years]; mean disease duration: 10.3 years [range 2–21, SD ± 5.6 years]). These 110 patients were of Western Finnish origin. Informed consent was obtained from the patients or their appropriate next of kin. Ethical approval for the study was given by the ethics review board of Turku University Hospital.

Blood-derived DNA samples were available from 47 early-onset PD patients. These patients originated from Southern and Eastern Finland (20 males, 27 females; mean age at onset 53 years). Informed consent was obtained from the patients or their appropriate next of kin. Ethical approval for the study was given by the ethics review board of Helsinki University Central Hospital and institutional review board of North Karelia Central Hospital.

2.2. Genetic analyses

In the postmortem cohort, DNAs were extracted from deep-frozen tissue using NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany). DNAs from blood samples were extracted with standard protocols. Exon 4 (the third coding exon) of *SNCA* was amplified by PCR using primers For: 5′-gctaatcagcaatttaaggctag-3′ and Rev: 5′-gatatgttcttagaatgctcag-3′. Purified PCR products were sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA).

The cohort of 47 PD patients was screened for mutations in PD loci by using HaloPlex targeted sequencing of 82 selected PD-associated loci (Agilent Technologies, Santa Clara, CA, USA). The sequencing was done with MiSeq sequencer (Illumina, San Diego, CA, USA). Variant calling was done with Genome Analysis Toolkit (McKenna et al., 2010) and the annotation with ANNOVAR (Wang et al., 2010).

Eight STR markers flanking a ~10-Mb area around *SNCA* were amplified by PCR using a 6-FAM-labeled reverse primer. PCR products were separated on an ABI 3730xl capillary sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed with the GeneMarker software (Softgenetics LLC, State College, PA, USA). In addition, all available samples from the 3 families were analyzed on a genome-wide SNP array (Human CoreExome BeadChip, Illumina, San Diego, CA, USA). Genotyping was performed by the Institute for Molecular Medicine Finland FIMM Technology Centre, University of Helsinki. Haplotype phases in the *SNCA* area and flanking regions were determined as stretches of SNP genotypes concordant for one or both alleles in patients from all the 3 families. Informative markers in parent-offspring duos in families F1 and F2 were used for phasing and the genotypes from patient F3 III:3 were compared with the phased genotypes.

3. Results

Sanger sequencing of *SNCA* exon 4 did not reveal any individuals with the p.Ala53Glu mutation in the cohort of 110 PD patients from Western Finland.

One new patient with the p.Ala53Glu mutation was identified in the cohort of 47 PD patients through targeted sequencing of 82 PD-associated genes. This patient presented with typical PD signs at the age of 41. At the age 43, the disease had progressed slowly and she did not require levodopa medication. She had dysphagia and dysarthria. [123I]beta-CIT SPECT imaging showed lowered dopamine transporter binding at the right putamen and caudate nucleus. Family history was compatible with autosomal dominant inheritance with at least 2 other affected family members (the index patient's mother and sister). Maternal grandmother may also have been affected by PD.

Haplotype analysis suggested that the patients with the p.Ala53Glu mutation share a common haplotype on chromosome 4. Phased STR marker haplotype in families F1 and F2 showed that the shared genomic segment spans the area between STR markers D4S2371 and D4S2380. The unphased STR markers of the singleton patient from family F3 had alleles consistent with this haplotype. Based on the physical locations of the markers, the shared region is at least 5.7 Mb in size, starting at marker D4S2371 (genomic location on chr4: 90,132,775) and extending to D4S2380 (genomic location on chr4: 95,883,055; Fig. 1). Analysis of the SNP data suggested breakages of the mutation-bearing haplotype at SNP rs2116325 (chr4: 90,115,197) and rs6842919 (chr4: 106,958,170) located 16.8 Mb from each other. The putative shared haplotype inferred from informative markers is shown in the Supplementary Table 1.

4. Discussion

Our results show that the *SNCA* p.Ala53Glu mutation is a rare cause of PD in the Finnish population. The mutation has been previously reported in 2 Finnish families originating from Western Finland, but no new patients with the mutation were identified in screening of 110 PD cases from Western Finland. One new case was identified in a family from Eastern Finland. In agreement with our results, no *SNCA* mutations were found in a cohort of 22 unrelated Eastern and Northern Finnish familial PD patients (Autere et al., 2002). The p.Ala53Glu mutation has not been reported in the 1000 Genomes, ExAC, ESP, or SISu databases (accessed June 2016).

The PD patients of the cohort from Western Finland were mostly sporadic, with mean age at onset of 60.4 years, whereas the patients from the cohort of 47 subjects were familial, with mean age at onset