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Brief communication

Analysis of *DNAJC13* mutations in French-Canadian/French cohort of Parkinson's disease

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ABSTRACT

DNAJC13 mutations have been suggested to cause Parkinson's disease (PD), yet subsequent studies reported conflicting results on this association. In the present study, we sequenced the coding region of *DNAJC13* in a French-Canadian/French cohort of 528 PD patients and 692 controls. A total of 62 (11.7%) carriers of rare *DNAJC13* variants were identified among the PD patients compared with 82 (11.8%) among controls ($p = 1.0$). Two variants that were previously suggested to be associated with PD, p.R1516H and p.L2170W, were identified with similar directions of association as previously reported. The p.R1516H was found in 2 (0.4%) patients versus 6 (0.9%, nonsignificant) controls and the p.L2170W variant was found in 9 (1.7%) patients and 5 (0.7%, nonsignificant) controls. Meta-analysis with previous reports resulted in odds ratios of 0.32 (95% confidence interval = 0.15–0.68, $p = 0.0037$) and 2.68 (95% confidence interval = 1.32–5.42, $p = 0.007$), respectively. Our results provide some support for the possibility that specific *DNAJC13* variants may play a minor role in PD susceptibility, although studies in additional populations are necessary.

1. Introduction

Recently, *DNAJC13* mutations have been implicated in the pathogenesis of PD. The *DNAJC13* gene is a possible source of risk factor variants and rare causal mutations (Appel-Cresswell et al., 2014; Gustavsson et al., 2015; Vilarino-Guell et al., 2014). A single variant in this gene, p.N855S, has been suggested to cause late-onset PD and has been demonstrated to cosegregate with PD within 5 families of Dutch–German–Russian Mennonite origin from Saskatchewan and British Columbia, Canada, albeit with incomplete penetrance and with phenocopies (Vilarino-Guell et al., 2014). An additional study suggested that other rare *DNAJC13* variants, such as p.R1516H, p.E1740Q, and p.L2170W, may be associated with increased or decreased risk for developing PD in Caucasian Canadian and Norwegian populations (Gustavsson et al., 2015). However, subsequent studies in large cohorts of Singapore Chinese and non-Hispanic-Caucasian populations did not identify mutations in *DNAJC13* associated with PD (Foo et al., 2014; Lorenzo-Betancor et al., 2015).

Although *DNAJC13* is a possible candidate gene for PD, the exact nature of the association remains inconclusive. To further examine the potential role of *DNAJC13* mutations in PD, we sequenced its coding regions in a cohort of French-Canadian and French PD patients and controls.

2. Methods

2.1. Study population

A cohort of unrelated and consecutively recruited 528 PD patients and 692 controls were included in this study. The cohort was

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composed of French-Canadian patients and controls from Quebec, collected at the Centre Hospitalier Universitaire de (CHU) de Quebec and Montreal Neurological Institute, Montreal, Canada, and from French patients and controls recruited at Montpellier, France. Average patient age was 65.7 ± 10.9 years, with 64.2% men. The control population included 2 groups, elderly controls (n = 105, average age at enrollment of 61.5 ± 7.6 years) and young controls (n = 576, average age at enrollment of 34.6 ± 6.4 years, data on age were not available for 11 controls). There was no significant difference in *DNAJC13* variants frequency between the 2 groups; therefore, we chose to analyze them as a single control population, with average age of 38.7 ± 11.8 years with 39.6% men. PD was diagnosed by movement disorder specialists according to the UK Brain Bank Criteria (Hughes et al., 1992), without excluding patients who have relatives with PD. All patients signed informed consent before entering the study, and the institutional review boards approved the study protocols.

2.2. *DNA extraction and* DNAJC13 *sequencing*

DNA was extracted using a standard salting out protocol. The coding sequence of *DNAJC13* was targeted using molecular inversion probes (MIPs), that were designed as described by O'Roak et al. (O'Roak et al., 2012). MIPs were selected based on their predicted quality, coverage, and overlap; and Supplementary Table 1 includes the MIPs used to sequence *DNAJC13* in the present study. The library was sequenced using the Illumina HiSeq 2500 platform at the McGill University and Génome Québec Innovation Centre. Sequence processing was done using Burrows-Wheeler Aligner (Li and Durbin, 2009), the Genome Analysis Toolkit (GATK, v.2.6.4) (McKenna et al., 2010), and ANNOVAR (Wang et al., 2010). Online prediction and conservation tools (SIFT(Kumar et al., 2009), PolyPhen 2 (Adzhubei et al., 2010), and GERP++(Davydov et al., 2010)) were used to assess the deleteriousness of the variants. Data on the frequency of each *DNAJC13* variant were extracted from public databases (Exome Aggregation Consortium, 1000 Genomes Project, NHLBI GO Exome Sequencing Project—Exome Variant Server). Only variants with high coverage and read quality were included in the analysis. All mutations that were analyzed were validated with Sanger sequencing in samples identified as carriers by MIPs.

2.3. *Statistical analysis*

The association between *DNAJC13* variants and PD was analyzed using a binary logistic regression with the status (patient or control) as a dependent variable, and age and sex as covariates. Because there was no difference in *DNAJC13* variant frequencies between the 2 control groups (elderly and young) and between men and women, we also used Fisher's exact test to examine the association between all *DNAJC13* mutations combined or private *DNAJC13* variants and PD. To perform a combined analysis of the p.R1516H and p.L2170W variants, we performed a meta-analysis by using an R package (Metafor; Wolfgang, 2010). Cochran-Mantel-Haenszel test was used to pool the studies and calculate the odds ratios (ORs) with a fixed-effect model. Tarone's test was applied to estimate heterogeneity. All other statistical analysis was performed using the SPSS, v2.1 software (IBM Inc).

3. Results

A total of 42 nonsynonymous variants in *DNAJC13* were found in patients and controls (Table 1), including 4 common variants (allele frequency >0.01 in our controls or in the 1000 Genomes Project and Exome Aggregation Consortium databases) and 38 rare variants. The combined frequency of rare variants was nearly identical in patients and controls (11.7% vs. 11.8%, respectively, OR = 0.99, 95% CI = 0.70–1.41, p = 1.0). The purported causal variant, p.N855S (Vilarino-Guell et al., 2014), was not identified in any of our cases or controls. Two variants that were previously reported to be associated with PD risk, p.R1516H and p.L2170W, were observed in 2 (0.4%) patients and 6 (0.9%) controls and 9 (1.7%) patients and 5 (0.7%) controls, respectively (Table 1, nonsignificant for both variants). Carriers of the p.R1516H variant had an average age of 55 for patients and 38.8 for controls, whereas carriers of the p.L2170W variant had an average age of 63 for patients and 41.8 for controls. A combined analysis of these 2 variants using both the present study and the different populations in the first study (Table 2) showed an OR of 0.32 (95%CI = 0.15–0.68, p = 0.0037, p for heterogeneity = 0.16) for p.R1516H and an OR of 2.68 (95%CI = 1.32–5.42, p = 0.007, p for heterogeneity = 0.83) for p.L2170W. We further examined whether variants that are predicted to be deleterious by both SIFT and PolyPhen 2 are associated with PD. Among PD patients, 53 (10.0%) carried such mutations and among controls 56 carried a predicted deleterious variant (8.1%, nonsignificant).

4. Discussion

None of the variants found in our cohort was independently associated with PD. However, 2 variants, p.L2170W and p.R1516H, had similar frequencies and risk direction as previously reported, and meta-analyses of these variants suggested that they may have a role in PD susceptibility. The meta-analysis included our population, and 3 populations that were previously published (Gustavsson et al., 2015). These populations included a discovery cohort of 201 PD patients and 194 controls from Canada, an additional population of 1042 PD patients and 497 controls from Canada, and 920 PD patients and 635 controls from Norway. The size of the current control population (n = 692) is larger than the published individual control populations, and our patient population (n = 528) is in-between the smallest and the largest PD populations, thus adding a substantial amount of patients and controls for the meta-analysis. Other studies in Caucasian (Lorenzo-Betancor et al., 2015) and Chinese (Foo et al., 2014) populations did not identify these variants because they genotyped specific exons or variants that did not include p.L2170W and p.R1516H, and therefore could not be included in the meta-analysis. The initially reported mutation, p.N855S, was not found in our cohort, suggesting that although this variant may be causative for PD in other ethnicities (Gustavsson et al., 2015), it is probably not an important cause of PD in French Canadians/French. The original family in which the p.N855S variant was identified was of Dutch–German–Russian Mennonite ancestry (Vilarino-Guell et al., 2014); it has been shown that p.N855S mutation is not a cause of PD in Caucasians (Lorenzo-Betancor et al., 2015), suggesting that even between similar ethnic groups, there could be differences in genetic causes of PD. In addition, our study identified several rare and novel variants in the *DNAJC13* gene. However, many of these were noninformative (only observed in 1 patient or control) or were not significantly associated with PD. Therefore, these variants need to be studied in additional populations to reach conclusions as to the role of *DNAJC13* in PD pathogenesis. A previous sequencing study of *GBA* mutations that included 212 patients and 190 controls from the present study was performed (Noreau et al., 2011), and none of the carriers of *GBA* mutations was a carrier of either p.L2170W or p.R1516H. Therefore, it is unlikely that *GBA* mutations had an effect on the present study results, and further studies are needed to determine if there is any association or interaction between *GBA* and *DNAJC13* in PD. The *DNAJC13* gene has previously been shown to be highly conserved across species (Girard et al., 2005; Gustavsson et al., 2015; Vilarino-Guell et al., 2014). Although this may suggest that variations in the