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Rare variants analysis of cutaneous malignant melanoma genes in Parkinson's disease

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ABSTRACT

A shared genetic susceptibility between cutaneous malignant melanoma (CMM) and Parkinson's disease (PD) has been suggested. We investigated this by assessing the contribution of rare variants in genes involved in CMM to PD risk. We studied rare variation across 29 CMM risk genes using high-quality genotype data in 6875 PD cases and 6065 controls and sought to replicate findings using whole-exome sequencing data from a second independent cohort totaling 1255 PD cases and 473 controls. No statistically significant enrichment of rare variants across all genes, per gene, or for any individual variant was detected in either cohort. There were nonsignificant trends toward different carrier frequencies between PD cases and controls, under different inheritance models, in the following CMM risk genes: *BAP1*, *DCC*, *ERBB4*, *KIT*, *MAPK2*, *MITF*, *PTEN*, and *TP53*. The very rare *TYR* p.V275F variant, which is a pathogenic allele for recessive albinism, was more common in PD cases than controls in 3 independent cohorts. Tyrosinase, encoded by *TYR*, is the rate-limiting enzyme for the production of neuromelanin, and has a role in the production of dopamine. These results suggest a possible role for another gene in the dopamine-biosynthetic pathway in susceptibility to neurodegenerative Parkinsonism, but further studies in larger PD cohorts are needed to accurately determine the role of these genes/variants in disease pathogenesis.

1. Introduction

Parkinson's disease (PD) is characterized by the progressive loss of postmitotic dopaminergic neurons, whereas cancer results from uncontrolled cellular proliferation. Although PD and cancer are

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distinct diseases, a relationship between PD and cancer is well established. Epidemiological studies have shown that although most cancers are less frequent in PD compared with the general population (Bajaj et al., 2010; Becker et al., 2010; Catalá-López et al., 2014; D'Amelio et al., 2004; Elbaz et al., 2002, 2005; Gao et al., 2009a; Kareus et al., 2012; Olsen et al., 2005, 2006; Ong et al., 2014; Wirdefeldt et al., 2014), cutaneous malignant melanoma (CMM) is found at an increased incidence in PD (Bajaj et al., 2010; Becker et al., 2010; Catalá-López et al., 2014; Kareus et al., 2012; Ong et al., 2014; Wirdefeldt et al., 2014). This well-documented association between CMM and PD is unexplained.

A genetic link between PD and CMM is supported by the demonstration of significant reciprocal risks of PD and CMM in cases and their relatives (Gao et al., 2009a, 2009b; Kareus et al., 2012). Although some support for a somatic genetic link between the 2 pathologies is provided by the role of Mendelian PD genes in CMM biology (Cesari et al., 2003; Kim et al., 2005; Liu et al., 2011; Matsuo and Kamitani, 2010; Millikin et al., 1991), there is currently no direct evidence for shared genetic susceptibility between PD and CMM.

Some studies have assessed the reciprocal role of common (minor allele frequency [MAF] $>$ 1%) genetic variation in CMM and PD. Recently, it has been suggested that the CMM-associated *MC1R* variants p.R151C and p.R160W increase PD risk but their role still remains unclear (Dong et al., 2014; Gao et al., 2009b; Lubbe et al., 2016; Tell-Marti et al., 2015). Previous studies using genome-wide association study variants associated with PD or CMM have failed to show any genetic overlap (Dong et al., 2014; Meng et al., 2012). More recently, rare de novo variants in the CMM risk gene *PTEN* have been implicated in PD (Kun-Rodrigues et al., 2015), but the role of rare coding variants underlying an association between PD and CMM has not yet been fully evaluated. Because the role of common genetic variation (variants with MAF $>$1%) has already been substantially addressed, we focused our investigation into the proposed shared genetic background between these diseases on rare variants (MAF $<$1%) in known CMM genes in 2 large independent PD case-control data sets as part of the International Parkinson's Disease Genomics Consortium.

2. Methods and materials

2.1. Genetic analysis

Using a systematic literature search, we identified susceptibility genes for CMM (Supplementary Table 1). These included (1) germline high-risk genes associated with familial CMM (e.g., *CDKN2A*, *CDK4*); (2) germline common moderate-risk genes (e.g., *MC1R*); (3) genes commonly somatically mutated (e.g., *BRAF*); and (4) recently identified genes found to harbor rare somatic mutations ascribed to CMM (e.g., *TRRAP*, *DCC*). Genes were selected based on defined roles in inherited high-penetrance autosomal dominant disease (n = 2); an excess of somatic mutations (n = 20); an excess of common low-penetrance risk variants (n = 3); or combinations of these (n = 4). All rare (MAF $<$1%) variants across these genes were assessed for enrichment in PD cases compared with unaffected controls.

We first assessed high-quality rare variant genotype data derived from the NeuroX chip on 6875 PD cases and 6065 controls (dbGaP Study Accession: phs000918.v1.p1). Briefly, the NeuroX chip has approximately 240,000 preselected variants based on standard Illumina exome content and over 24,000 custom content neurologic disease focused variants (Nalls et al., 2015).

We next assessed whole-exome sequencing data on 1255 PD cases and 473 controls from the International Parkinson's Disease Genomics Consortium. Briefly, sample libraries from cases and controls were prepared using either Roche Nimblegen (cases, n = 334; controls, n = 40) or Illumina (cases, n = 921; controls, n = 433) capture kits with paired-end sequencing performed on the Illumina HiSeq2000. Reads were aligned using Burrows-Wheeler Aligner (Li and Durbin, 2009) against the University of California Santa Cruz (UCSC) hg19 reference genome. Variant calling and quality-based filtering were done using Genome Analysis Tool Kit (GATK) (McKenna et al., 2010). ANNOVAR (Wang et al., 2010) was used to annotate variants with predicted impact of variants from the following in silico tools: SIFT (Ng and Henikoff, 2001), PhyloP (Pollard et al., 2010), PolyPhen-2 (Adzhubei et al., 2010), LRT (Chun and Fay, 2009), MutationTaster (Schwarz et al., 2010), and GERP++ (Davydov et al., 2010).

Of the 29 identified CMM genes, only 24 were represented on the NeuroX panel (Supplementary Table 1). Based on the annotated MAF data from 1000 Genomes Project (http://www.1000genomes.org/) and NHLBI GO Exome Sequencing Project (https://evs.gs.washington.edu/EVS/), all rare variants (MAF $<$ 1%) were extracted and assessed in PD cases and controls. We defined the potential deleterious impact of variants using previously defined methods (Fu et al., 2013; Tennessen et al., 2012) with variants classified as damaging if $\geq$4 of the 6 in silico tools used predicted the change deleterious. Variants and samples with $>$5% missing calls were excluded during QC.

All exome generated FastQs were run through the same pipeline and merged to generate high-quality genotype data. Damaging variants were defined as stated above. The GATK recommended filtering of variants, including the removal of variants with low coverage (read depth $<$5), was implemented over and above the QC stated above. Post QC, 28 of the 29 selected CMM genes were covered by one or both captures methods (Supplementary Table 1), and no difference between capture methods was observed with majority of all exons represented and included in the analyses (Supplementary Table 2).

Candidate variants were also assessed in high-quality exome sequencing data generated from a CMM case-control cohort (CMM, n = 1298; Controls, n = 684) to investigate any reciprocal risks for CMM.

2.2. Statistical analysis

SNP-Set (Sequence) Kernel Association Test (SKAT) (Wu et al., 2011) was used to test for association between the rare variants in genes and PD (gene- and gene set-based), adjusting for covariates including gender, coverage metrics and principal components (1–4). Dominant and recessive models of inheritance for each CMM gene were modeled and assessed using STATA (version 10; STATA, State College, TX, USA) via logistic regression, adjusting for covariates. For variants common to both cohorts, meta-analyses were conducted using standard methods modeling fixed effects (Petitti, 1994). Cochran's Q-statistic was calculated to test for heterogeneity (P_{het}) (Petitti, 1994), and the I^2 statistic (Higgins and Thompson, 2002) was generated to quantify the proportion of the total variation caused by heterogeneity. Bonferroni's correction was applied, where applicable, to account for multiple testing.

3. Results

3.1. Rare variant screening and burden analysis

The NeuroX data contained 237 variants with $\geq$1 nonreference allele after QC, including 215 (90.7%) nonsynonymous single nucleotide polymorphisms (nsSNPs), and 17 (7.2%) loss of function (LOF) variants (stop gains or losses, splice, frame- or nonframeshift indels). About 554 variants with $\geq$1 nonreference allele were