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Evaluation of novel Parkinson's disease candidate genes in the Chinese population

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ABSTRACT

Recent whole-exome sequencing studies in European patients with Parkinson's disease (PD) have identified potential risk variants across 33 novel PD candidate genes. We aim to determine if these reported candidate genes are similarly implicated in Asians by assessing common, rare, and novel nonsynonymous coding variants by sequencing all 33 genes in 198 Chinese samples and genotyping coding variants in an independent set of 9756 Chinese samples. We carried out further targeted sequencing of *CD36* in an additional 576 Chinese and Korean samples. We found that only 8 of 43 reported risk variants were polymorphic in our Chinese samples. We identified several heterozygotes for rare loss-of-function mutations, including the reported *CD36* p.Gln74Ter variant, in both cases and controls. We also observed 2 potential compound heterozygotes among PD cases for rare loss-of-function mutations in *CD36* and *SSPO*. The other reported variants were common in East Asians and not associated with PD, completely absent, or only found in controls. Therefore, the 33 reported candidate genes and associated variants are unlikely to confer significant PD risk in the East Asian population.

1. Introduction

Two recent whole-exome sequencing (WES) studies in patients with Parkinson's disease (PD) and controls of Finnish (Siitonen et al., 2017) and European (Jansen et al., 2017) descent have identified a total of 33 novel candidate genes for early-onset PD, containing either low-penetrance risk variants (Siitonen et al., 2017) or potentially high-penetrance recessive disease variants from mostly single observations of homozygotes or compound heterozygotes for loss-of-function (LoF) mutations (Jansen et al., 2017). It is unknown if these variants are present in the Asian population as well. A comparison of the allelic spectrum of these genes in Asian compared with European populations can provide insight into the pathogenicity of these variants and the genetic differences underlying PD in these populations.

2. Methods

We assessed if these 33 genes are implicated in PD risk in the Asian population through sequencing and genotyping of 9954 ethnic Chinese samples. Specifically, 9 of the 43 reported variants and 146 common nonsynonymous coding variants (minor allele

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Table 1
Loss-of-function mutations identified in 99 cases and 99 controls from the Chinese population across 33 potential novel PD candidate genes

	Gene	HGVS nomenclature (Hg19)	Amino acid substitution[a]	Variant	gnomAD allele frequency		Case				Control			
					Average	East Asian	MAF	All[b]	Het[c]	Homo rec[d]	MAF	All[b]	Het[c]	Homo rec[d]
Stop-gain	*CAPS2*	NC_000012.11:g.75687069G>A	p.Arg394Ter	rs201447508	5.80E-05	5.32E-05	0.0051	1	1	0	0	0	0	0
	CD36	NC_000007.13:g.80285955C>T	p.Gln74Ter	rs545489204	0.00015	0.00074	0.0051	1	1	0	0	0	0	0
	MNS1	NC_000015.9:g.56721299T>C	p.Ter496TrpextTer16	rs147344343	1.75E-05	0.00012	0.0051	1	1	0	0	0	0	0
	PTPRH	NC_000019.9:g.55710183G>C	p.Tyr328Ter	rs775533113	2.5E-05	0.00035	0.0051	1	1	0	0	0	0	0
	SSPO	NC_000007.13:g.149480366C>T	p.Gln85Ter	rs540871992	0.00016	0.00228	0.0051	1	1	0	0	0	0	0
	SSPO	NC_000007.13:g.149499259C>T	p.Arg2540Ter	rs138029458	0.00025	0.00236	0.0051	1	1	0	0	0	0	0
	SSPO	NC_000007.13:g.149509455C>T	p.Gln3282Ter	rs529382220	0.00028	0.00325	0.0051	1	1	0	0.0051	1	1	0
	SSPO	NC_000007.13:g.149518093C>T	p.Gln4143Ter	novel	NA	NA	0.0051	1	1	0	0	0	0	0
Frameshift	*ANKRD36*	NC_000002.11:g.97907101_97907102insA	p.Arg1424GlnfsTer6	NA	NA	NA	0.0101	2	2	0	0.0152	2	1	1
	ANKRD36	NC_000002.11:g.97909603_97909607delAAAAG	p.Arg1472LysfsTer3	NA	0.00252	0.03866	0.0404	8	8	0	0.0455	9	9	0
	CD36	NC_000007.13:g.80290426_80290427delAC	p.Thr111SerfsTer22	rs572295823	0.00105	0.01514	0.0202	4	4	0	0.0051	1	1	0
	CD36	NC_000007.13:g.80303371_80303372insTGAT	p.Glu445AspfsTer65	rs750173933	3.63E-05	0.00037	0.0051	1	1	0	0	0	0	0
	OR7G3	NC_000019.9:g.9236703_9236704insATGGC	p.Ile308MetfsTer?	rs111279560	1.66E-05	5.81E-5	0.0859	13	9	4	0.0606	9	6	3
	PTCHD3	NC_000010.10:g.27702261_27702262insG	p.Gly307ArgfsTer28	rs148676840	0.00052	0.00721	0.0303	6	6	0	0.0303	5	4	1
	PTPRH	NC_000019.9:g.55697680_55697681insGA	p.Gln720HisfsTer22	NA	3.24E-05	0.00062	0.0051	1	1	0	0	0	0	0
Splice site	*ANKRD36*	NC_000002.11:g.97823843G>C	NA	NA	NA	NA	0.2727	54	54	0	0.3232	64	64	0
	PZP	NC_000012.11:g.9353922C>T	NA	rs200164577	0.00016	0.00212	0.0101	2	2	0	0	0	0	0
	SSPO	NC_000007.13:g.149477219G>A	p.Glu466Lys	rs568165115	0.00027	0.00227	0.0101	2	2	0	0	0	0	0
	SSPO	NC_000007.13:g.149482828G>A	p.Gly1082Ser	rs543893638	0.00013	0.00188	0.0051	1	1	0	0	0	0	0

[a] Amino acids affected by frameshift mutation are indicated by positional range of amino acids.
[b] Number of case/control samples whose variant was identified.
[c] Number of case/control heterozygotes whose variant was identified.
[d] Number of case/control recessive homozygotes whose variant was identified.

frequency (MAF) > 1%) were genotyped in 710 late-onset (>55 years) Chinese PD cases and 9046 population controls, whereas rare and novel nonsynonymous coding variants (MAF ≤ 1%) were identified by WES of 99 patients with early-onset PD (<55 years) and 99 healthy elderly controls. *CD36* targeted sequencing was conducted in another 576 Chinese and Korean samples (276 early-onset cases, 300 controls). These samples have previously been assessed for mutations/variants in established familial PD genes and other PD-associated genes (Foo et al., 2014). Patients were diagnosed with PD using the UK Brain Bank criteria. All patients and controls gave informed written consent, and the study was approved by the institutional ethics committee (Supplementary Methods).

3. Results and discussion

Of the 43 reported variants, only 10 were polymorphic in Exome Aggregation Consortium (ExAC) East Asians and 8 were polymorphic in our Chinese samples (Supplementary Table 1), underscoring the intrinsic difficulty of validating WES data across populations. Most of the reported low-penetrance risk variants (Siitonen et al., 2017) are rare in Europeans and absent in East Asians, with the exception of *TAS2R19* p.Lys126Gln that is common in East Asians (allele frequency ~12%) and not significantly associated with PD in our samples. Other common nonsynonymous coding variants observed within the reported genes also showed no association with PD (Supplementary Table 2). Among the reported high-penetrance risk variants (Jansen et al., 2017), 5 were common in East Asians, whereas the rare *DIS3* and *GPATCH2L* variants were found solely in controls (Supplementary Table 1). These variants are unlikely to be major PD risk factors in Chinese. The *CD36* p.Gln74Ter variant, although not significantly enriched in cases, was present at higher frequencies in cases (0.51% early-onset and 0.28% late-onset cases) than in controls (0.18%).

From our WES data, rare nonsynonymous variants we identified across 31 candidate PD genes were not significantly enriched in cases or controls (Supplementary Table 3). We identified possible compound heterozygotes in 1/99 cases each for *CD36* and *SSPO* (Supplementary Table 4). Further targeted sequencing of *CD36* did not identify any additional compound heterozygotes or homozygotes for this or other variants in *CD36* (Supplementary Table 5, Supplementary Fig. 1). This confirmed that, akin to the initial discovery European population (1/1148 early-onset PD cases) (Jansen et al., 2017), compound heterozygotes or homozygotes for LoF mutations in PD risk genes are similarly rare in early-onset Asian PD cases. We observed 23 predicted LoF variants, including 10 stop-gain, 9 frameshifts, and 4 splice site variants in 9 genes (Table 1). We observed 3 homozygotes for LoF variants among healthy controls, all of which appear to be low-frequency polymorphisms in East Asian populations and unlikely to play a major role in health and disease (Lek et al., 2016; MacArthur et al., 2012). The heterozygous LoF variants present only in cases but not controls are potential PD risk variants that require further validation.

In our current samples, we have 80% estimated power ($\alpha = 0.05$) to identify genes with high-penetrant rare variants (Jansen et al., 2017) in at least 8% (8/99 cases, Supplementary Figure 2) and 0.43% (3/710 cases, Supplementary Fig. 3) of our early- and late-onset cases, respectively. We did not observe this occurrence of heterozygotes or homozygotes/compound heterozygotes in any of the 33 genes, suggesting they are unlikely to contribute significantly to PD risk in our Chinese patients. Although the rare reported variants were originally found in patients with early-onset PD (Jansen et al., 2017; Siitonen et al., 2017), we observed most of them at similar frequencies in our early- and late-onset PD samples, suggesting that age-related differences are unlikely.

Of note, the previous WES studies (Jansen et al., 2017; Siitonen et al., 2017) also did not observe significant enrichment of these extremely rare variants and encountered limitations in power to study these rare variants, with reported detection power (Siitonen et al., 2017) ranging from 5.3×10^{-7} to 1.4×10^{-4}. We estimate that at least 10,597 early-onset PD cases and controls are required for robust detection of the reported rare variants (80% power, $\alpha = 0.05$; case:control = 1:1). Analyzing segregation in families may be an alternative to validate these rare variants in the Chinese population, although such families are limited for a late-onset disease such as PD. Ultimately, large international consortia need to be formed for meta-analyses across multiple sample collections.

It is possible that some of the reported novel rare PD variants are population-specific and thus were not detected at all in our Chinese PD samples. There are several examples of population-specific rare variants, including the PD pathogenic mutation *LRRK2* p.Gly2019Ser (Kumari and Tan, 2009) and the Alzheimer's disease risk variant *TREM2* p.Arg47His (Huang et al., 2015), each of which is consistently associated with disease risk in Europeans and other populations but resolutely absent in Asians across multiple studies. It is also possible that certain reported rare variants play a stronger role in early-onset PD as opposed to late-onset PD. However, for the majority of the reported rare variants interrogated in our samples, we observed similar frequencies of interrogated variants in our late-onset samples (710 PD cases with onset >55 years) as compared with our early-onset samples (99 PD cases with onset <55 years) (Supplementary Table 1).

Nonetheless, the identification of potential compound heterozygotes for mutations in *CD36* and *SSPO* in additional PD cases provides support for their possible roles in PD pathogenesis. *CD36* is known to have a role in malaria by mediating cytoadherence of *Plasmodium falciparum*–parasitized erythrocytes (Oquendo et al., 1989). Given that anti-malarial therapy has been previously observed to alleviate PD-associated dyskinesia in a rat model (Kim et al., 2015), this may represent an indirect potential functional link between *CD36* and PD. The exact role of *SSPO* in PD pathogenicity is undetermined; however, *SSPO* is involved in neuronal survival (Monnerie et al., 1997) and aggregation (Gobron et al., 1996), neurite extension (Gobron et al., 2000), as well as fasciculation (Meiniel et al., 2003; Stanic et al., 2010).

4. Conclusion

In summary, the majority of reported PD risk genes and associated variants are either extremely rare or absent in our Chinese samples and thus unlikely to confer significant PD risk in the Chinese population. There are more than 1.5 billion Chinese globally, accounting for approximately 20% of the global population. Our findings in the Chinese population are therefore an important contribution to the literature for future reference and meta-analysis. Further sequencing in substantially larger data sets from diverse populations is required to determine if these recessive mutations are enriched in early-onset PD cases and/or have population-specific effects. Longitudinal evaluation of both patients and healthy controls who carry the reported and identified rare LoF variants, with clinical and neuroimaging assessments, will allow in vivo elucidation of their pathogenicity in humans.

Disclosure statement

The authors have no conflicts of interest to declare.

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Data access

Data are available in Supplementary Material and upon request at https://www.nni.com.sg/research/research-platforms/Genomics/Pages/Home.aspx.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2018.09.013.

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