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

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Abstract (178 words)

Recent whole exome sequencing studies in European Parkinson's disease (PD) patients 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 non-synonymous coding variants by sequencing all 33 genes in 198 Chinese samples, and genotyping coding variants in an independent set of 9,756 Chinese samples. We carried out further targeted sequencing of *CD36* in additional 576 Chinese and Korean samples. We found that only 8 out 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 two potential compound heterozygotes amongst PD cases for rare loss-of-function mutations in *CD36* and *SSPO*. The other reported variants were either 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.

Key words: Parkinson's disease, Genetics, Chinese population

1. Introduction

Two recent whole exome sequencing (WES) studies in Parkinson's disease (PD) patients 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 to 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 9,954 ethnic Chinese samples. Specifically, 9 of the 43 reported variants and 146 common non-synonymous coding variants ($MAF > 1\%$) were genotyped in 710 later-onset Chinese PD cases and 9,046 population controls, while rare and novel non-synonymous coding variants ($MAF \leq 1\%$) were identified by WES of 99 early-onset PD patients 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 using the UK Brain Bank criteria. All patients and controls gave informed written consent and study was approved by the institutional ethics committee (**Supplementary Methods**).

3. Results and Discussion

Out of the 43 reported variants, only 10 were polymorphic in 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 which is common in East Asians (allele frequency ~12%) and not significantly associated with PD in our samples. Other common non-synonymous 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), five were common in East Asians while 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-, 0.28% later-onset cases) than in controls (0.18%).

From our WES data, rare non-synonymous 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 Figure 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 three 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 diseases (Lek et al., 2016; MacArthur et al., 2012). The heterozygous LoF variants present only in cases but not controls are potential PD risk variants which require further validation.

In our current samples, we have 80% estimated power ($\alpha=0.05$) to identify genes with highly-penetrant rare variants (Jansen *et al.*, 2017) in at least 8% (8/99 cases, **Supplementary Figure 2**) and 0.43% (3/710 cases, **Supplementary Figure 3**) of our early- and later-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. Even though the rare reported variants were originally found in early-onset PD patients (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 like PD. Ultimately, large international consortiums 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 was 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 later-onset PD. However, for the majority of the reported rare variants

interrogated in our samples, we observed similar frequencies of interrogated variants in our later-onset samples (710 PD cases with onset >55 years) as compared to 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 provide 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-malaria therapy has been previously observed to alleviate PD-associated dyskinesia in 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 gene 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 is therefore an important contribution to the literature for future reference and meta-analysis. Further sequencing in substantially larger datasets 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 its pathogenicity in humans.

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Data Access

Data is available in supplementary material and upon request at

<https://www.nni.com.sg/research/research-platforms/Genomics/Pages/Home.aspx>

References

- Foo, J.N., Tan, L.C., Liany, H., Koh, T.H., Irwan, I.D., Ng, Y.Y., Ahmad-Annuar, A., Au, W.L., Aung, T., Chan, A.Y., Chong, S.A., Chung, S.J., Jung, Y., Khor, C.C., Kim, J., Lee, J., Lim, S.Y., Mok, V., Prakash, K.M., Song, K., Tai, E.S., Vithana, E.N., Wong, T.Y., Tan, E.K., Liu, J., 2014. Analysis of non-synonymous-coding variants of Parkinson's disease-related pathogenic and susceptibility genes in East Asian populations. *Hum Mol Genet* 23(14), 3891-3897.
- Gobron, S., Creveaux, I., Meiniel, R., Didier, R., Herbet, A., Bamdad, M., El Bitar, F., Dastugue, B., Meiniel, A., 2000. Subcommissural organ/Reissner's fiber complex: characterization of SCO-spondin, a glycoprotein with potent activity on neurite outgrowth. *Glia* 32(2), 177-191.
- Gobron, S., Monnerie, H., Meiniel, R., Creveaux, I., Lehmann, W., Lamalle, D., Dastugue, B., Meiniel, A., 1996. SCO-spondin: a new member of the thrombospondin family secreted by the subcommissural organ is a candidate in the modulation of neuronal aggregation. *Journal of Cell Science* 109(5), 1053.
- Huang, M., Wang, D., Xu, Z., Xu, Y., Xu, X., Ma, Y., Xia, Z., 2015. Lack of genetic association between TREM2 and Alzheimer's disease in East Asian population: a systematic review and meta-analysis. *American journal of Alzheimer's disease and other dementias* 30(6), 541-546.
- Jansen, I.E., Ye, H., Heetveld, S., Lechler, M.C., Michels, H., Seinstra, R.I., Lubbe, S.J., Drouet, V., Lesage, S., Majounie, E., Gibbs, J.R., Nalls, M.A., Ryten, M., Botia, J.A., Vandrovcova, J., Simon-Sanchez, J., Castillo-Lizardo, M., Rizzu, P., Blauwendraat, C., Chouhan, A.K., Li, Y., Yogi, P., Amin, N., van Duijn, C.M., Morris, H.R., Brice, A., Singleton, A.B., David, D.C., Nollen, E.A., Jain, S., Shulman, J.M., Heutink, P., 2017. Discovery and functional prioritization of Parkinson's disease candidate genes from large-scale whole exome sequencing. *Genome Biol* 18(1), 22.
- Kim, C.H., Han, B.S., Moon, J., Kim, D.J., Shin, J., Rajan, S., Nguyen, Q.T., Sohn, M., Kim, W.G., Han, M., Jeong, I., Kim, K.S., Lee, E.H., Tu, Y., Naffin-Olivos, J.L., Park, C.H., Ringe, D., Yoon, H.S., Petsko, G.A., Kim, K.S., 2015. Nuclear receptor Nurr1 agonists enhance its dual functions and improve behavioral deficits in an animal model of Parkinson's disease. *Proc Natl Acad Sci U S A* 112(28), 8756-8761.
- Kumari, U., Tan, E.K., 2009. LRRK2 in Parkinson's disease: genetic and clinical studies from patients. *The FEBS journal* 276(22), 6455-6463.
- Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., O'Donnell-Luria, A.H., Ware, J.S., Hill, A.J., Cummings, B.B., Tukiainen, T., Birnbaum, D.P., Kosmicki, J.A., Duncan, L.E., Estrada, K., Zhao, F., Zou, J., Pierce-Hoffman, E., Berghout, J., Cooper, D.N., Deflaux, N., DePristo, M., Do, R., Flannick, J., Fromer, M., Gauthier, L., Goldstein, J., Gupta, N., Howrigan, D., Kiezun, A., Kurki, M.I., Moonshine, A.L., Natarajan, P., Orozco, L., Peloso, G.M., Poplin, R., Rivas, M.A., Ruano-Rubio, V., Rose, S.A., Ruderfer, D.M., Shakir, K., Stenson, P.D., Stevens, C., Thomas, B.P., Tiao, G., Tusie-Luna, M.T., Weisburd, B., Won, H.H., Yu, D., Altshuler, D.M., Ardissino, D., Boehnke, M., Danesh, J., Donnelly, S., Elosua, R., Florez, J.C., Gabriel, S.B., Getz, G., Glatt, S.J., Hultman, C.M., Kathiresan, S., Laakso, M., McCarroll, S., McCarthy, M.I., McGovern, D., McPherson, R., Neale, B.M., Palotie, A., Purcell, S.M., Saleheen, D., Scharf, J.M., Sklar, P., Sullivan, P.F., Tuomilehto, J., Tsuang, M.T., Watkins, H.C., Wilson, J.G., Daly, M.J., MacArthur, D.G., 2016. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536(7616), 285-291.
- MacArthur, D.G., Balasubramanian, S., Frankish, A., Huang, N., Morris, J., Walter, K., Jostins, L., Habegger, L., Pickrell, J.K., Montgomery, S.B., Albers, C.A., Zhang, Z.D., Conrad, D.F., Lunter, G., Zheng, H., Ayub, Q., DePristo, M.A., Banks, E., Hu, M., Handsaker, R.E., Rosenfeld, J.A., Fromer, M., Jin, M., Mu, X.J., Khurana, E., Ye, K., Kay, M., Saunders, G.I., Suner, M.-M., Hunt, T., Barnes, I.H.A., Amid, C., Carvalho-Silva, D.R., Bignell, A.H., Snow,

- C., Yngvadottir, B., Bumpstead, S., Cooper, D.N., Xue, Y., Romero, I.G., Wang, J., Li, Y., Gibbs, R.A., McCarroll, S.A., Dermitzakis, E.T., Pritchard, J.K., Barrett, J.C., Harrow, J., Hurles, M.E., Gerstein, M.B., Tyler-Smith, C., 2012. A Systematic Survey of Loss-of-Function Variants in Human Protein-Coding Genes. *Science* 335(6070), 823.
- Meiniel, A., Meiniel, R., Gonçalves-Mendes, N., Creveaux, I., Didier, R., Dastugue, B., 2003. The Thrombospondin Type 1 Repeat (TSR) and Neuronal Differentiation: Roles of SCO-Spondin Oligopeptides on Neuronal Cell Types and Cell Lines*, *International Review of Cytology*. Academic Press, pp. 1-39.
- Monnerie, H., Dastugue, B., Meiniel, A., 1997. In vitro differentiation of chick spinal cord neurons in the presence of Reissner's fibre, an ependymal brain secretion. *Brain Res Dev Brain Res* 102(2), 167-176.
- Oquendo, P., Hundt, E., Lawler, J., Seed, B., 1989. CD36 directly mediates cytoadherence of Plasmodium falciparum parasitized erythrocytes. *Cell* 58(1), 95-101.
- Siitonen, A., Nalls, M.A., Hernandez, D., Gibbs, J.R., Ding, J., Ylikotila, P., Edsall, C., Singleton, A., Majamaa, K., 2017. Genetics of early-onset Parkinson's disease in Finland: exome sequencing and genome-wide association study. *Neurobiol Aging* 53, 195.e197-195.e110.
- Stanic, K., Montecinos, H., Caprile, T., 2010. Subdivisions of chick diencephalic roof plate: implication in the formation of the posterior commissure. *Developmental dynamics : an official publication of the American Association of Anatomists* 239(10), 2584-2593.

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
<i>Stop-gain</i>	<i>CAPS2</i>	NC_000012.11:g.75687069G>A	p.Arg394Ter	rs201447508	5.80E-05	5.32E-05	0.0051	1	1	0	0	0	0	0
	<i>CD36</i>	NC_000007.13:g.80285955C>T	p.Gln74Ter	rs545489204	0.00015	0.00074	0.0051	1	1	0	0	0	0	0
	<i>MNS1</i>	NC_000015.9:g.56721299T>C	p.Ter496TrpextTer16	rs147344343	1.75E-05	0.00012	0.0051	1	1	0	0	0	0	0
	<i>PTPRH</i>	NC_000019.9:g.55710183G>C	p.Tyr328Ter	rs775533113	2.5E-05	0.00035	0.0051	1	1	0	0	0	0	0
	<i>SSPO</i>	NC_000007.13:g.149480366C>T	p.Gln85Ter	rs540871992	0.00016	0.00228	0.0051	1	1	0	0	0	0	0
	<i>SSPO</i>	NC_000007.13:g.149499259C>T	p.Arg2540Ter	rs138029458	0.00025	0.00236	0.0051	1	1	0	0	0	0	0
	<i>SSPO</i>	NC_000007.13:g.149509455C>T	p.Gln3282Ter	rs529382220	0.00028	0.00325	0.0051	1	1	0	0.0051	1	1	0
	<i>SSPO</i>	NC_000007.13:g.149518093C>T	p.Gln4143Ter	novel	NA	NA	0.0051	1	1	0	0	0	0	0
<i>Frameshift</i>	<i>ANKRD36</i>	NC_000002.11:g.97907101_97907102insA	p.Arg1424GlnfsTer6	NA	NA	NA	0.0101	2	2	0	0.0152	2	1	1
	<i>ANKRD36</i>	NC_000002.11:g.97909603_97909607delAAAAG	p.Arg1472LysfsTer3	NA	0.00252	0.03866	0.0404	8	8	0	0.0455	9	9	0
	<i>CD36</i>	NC_000007.13:g.80290426_80290427delAC	p.Thr111SerfsTer22	rs572295823	0.00105	0.01514	0.0202	4	4	0	0.0051	1	1	0
	<i>CD36</i>	NC_000007.13:g.80303371_80303372insTGAT	p.Glu445AspfsTer65	rs750173933	3.63E-05	0.00037	0.0051	1	1	0	0	0	0	0
	<i>OR7G3</i>	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
	<i>PTCHD3</i>	NC_000010.10:g.27702261_27702262insG	p.Gly307ArgfsTer28	rs148676840	0.00052	0.00721	0.0303	6	6	0	0.0303	5	4	1
	<i>PTPRH</i>	NC_000019.9:g.55697680_55697681insGA	p.Gln720HisfsTer22	NA	3.24E-05	0.00062	0.0051	1	1	0	0	0	0	0
<i>Splice site</i>	<i>ANKRD36</i>	NC_000002.11:g.97823843G>C	NA	NA	NA	NA	0.2727	54	54	0	0.3232	64	64	0
	<i>PZP</i>	NC_000012.11:g.9353922C>T	NA	rs200164577	0.00016	0.00212	0.0101	2	2	0	0	0	0	0
	<i>SSPO</i>	NC_000007.13:g.149477219G>A	p.Glu466Lys	rs568165115	0.00027	0.00227	0.0101	2	2	0	0	0	0	0
	<i>SSPO</i>	NC_000007.13:g.149482828G>A	p.Gly1082Ser	rs543893638	0.00013	0.00188	0.0051	1	1	0	0	0	0	0

Note: a - amino acids affected by frameshift mutation is indicated by positional range of amino acids; b - Number of case/control samples which variant was identified; c - Number of case/control heterozygotes which variant was identified; d - Number of case/control recessive homozygotes which variant was identified.