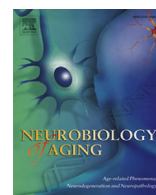




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## Brief communication

Lack of evidence for a role of genetic variation in *TMEM230* in the risk for Parkinson's disease in the Caucasian population

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## ABSTRACT

Mutations in *TMEM230* have recently been associated to Parkinson's disease (PD). To further understand the role of this gene in the Caucasian population, we interrogated our large repository of next generation sequencing data from unrelated PD cases and controls, as well as multiplex families with autosomal dominant PD. We identified 2 heterozygous missense variants in 2 unrelated PD cases and not in our control database (p.Y106H and p.I162V), and a heterozygous missense variant in 2 PD cases from the same family (p.A163T). However, data presented herein is not sufficient to support the role of any of these variants in PD pathology. A series of unified sequence kernel association tests also failed to show a cumulative effect of rare variation in this gene on the risk of PD in the general Caucasian population. Further evaluation of genetic data from different populations is needed to understand the genetic role of *TMEM230* in PD etiology.

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<sup>2</sup> A complete list of the IPDGC members is listed in the [Supplementary Data](#).

## 1. Introduction

In a recent publication, Deng et al. showed genetic evidence linking a mutation in transmembrane protein 230 (*TMEM230*) to autosomal dominant Parkinson's disease (PD) in a large family from North America (Deng et al., 2016). Identification of an additional mutation in 7 small Chinese families supports their results and suggests that mutations in this gene may be a relatively common cause of PD in this population. They also showed that *TMEM230* encodes a transmembrane protein of synaptic vesicles in neurons and that disease-linked mutations impair synaptic vesicle trafficking. Presence of *TMEM230* in alpha-synuclein-positive Lewy bodies and Lewy neurites in midbrain and neocortex sections from sporadic PD cases gave supporting evidence for a role of this gene in PD pathology (Deng et al., 2016).

In an effort to understand the role of *TMEM230* mutations in the general Caucasian population, the authors also screened a larger cohort of 433 familial and 399 sporadic PD cases. This led to the identification of 2 additional variants in 2 PD cases from North America (Deng et al., 2016). Because these variants were identified in a single PD patient each, their pathogenicity—and consequently the role of mutations in *TMEM230* in this population—remains to be further evaluated.

To further investigate the putative pathogenicity of *TMEM230* mutations in the Caucasian population, we interrogated our large repository of next-generation sequencing data from unrelated PD cases and controls, as well as multiplex families with autosomal dominant PD.

## 2. Subjects and methods

### 2.1. IPDGC and Rotterdam Study Exome Sequencing Database (*RSX1*) cohorts

To establish the role of *TMEM230* in the general Caucasian population, we mined our large repository of whole exome sequencing (WES) data consisting of 1450 PD cases and 535 controls from the International Parkinson's Disease Consortium (IPDGC), and 1732 controls from the *RSX1* (Hofman et al., 2013, 2015; Ikram et al., 2011). After genotype and variant quality control (Supplementary Methods), we were left with a total of 61 variants spanning *TMEM230* of which only 10 had a direct impact on the coding sequence of this gene. Only 2 of these variants were present in PD cases only: NM\_001009923: c.T316C: p.Y106H and NM\_001009923: c.A484G: p.I162V (Table 1).

After a more extensive quality control procedure that included genotype, variant, and individual filtering, this data set was also used to perform a series of unified sequence kernel association

tests (SKAT-O) aiming to understand the cumulative impact of rare variation (minor allele frequency [MAF]  $\leq$  0.01) in *TMEM230* on the risk of PD (Lee et al., 2012). For this analysis, raw combined annotation dependent depletion scores (<http://cadd.gs.washington.edu/>) (Kircher et al., 2014) were used as custom weights for each variant, whereas gender, mean sequencing depth, and the first 20 components derived from multidimensional scale analysis were used as covariates (Supplementary Methods).

### 2.2. Multiplex families with autosomal dominant PD

In an effort to replicate the main finding described by Deng et al., we also mined WES and whole genome sequencing data from 29 multiplex families with autosomal dominant PD originally from France, Spain, Portugal, the Netherlands, and the UK (Supplementary Table S1). For each of these families, data on at least 2 PD cases were available. In total, data from 86 PD cases and 10 controls were analyzed. For details on the quality control procedures applied see the Supplementary Methods.

## 3. Results and discussion

To investigate the putative pathogenicity of *TMEM230* mutations in the general Caucasian population, we interrogated our large repository of WES data from unrelated PD cases and controls (1450 and 2267, respectively) from the IPDGC and the Rotterdam Study *RSX1* (Hofman et al., 2013, 2015; Ikram et al., 2011). This analysis failed to identify any of the pathogenic mutations described by Deng et al. although this locus was sequenced at an average depth of  $\sim$ 30X, suggesting that these variants are very rare. Further investigation of our WES data identified 2 rare heterozygous missense variants (NM\_001009923: c.T316C: p.Y106H and NM\_001009923: c.A484G: p.I162V) present in 2 North American PD cases and not present in any of the control samples investigated ( $p = 0.37$  and  $0.38$  after Fisher's exact test; Table 1). Both carriers suffer of bradykinesia, activation tremor, resting tremor, rigidity and were classified as nonfamilial. The variants identified had 1 and 2 alleles, respectively, in European (non-Finnish) ExAC population (MAF = 0.00001501 and 0.00002997) and were predicted to be pathogenic as per phred combined annotation dependent depletion scores of 24 and 22.2 (Amendola et al., 2015; Kircher et al., 2014), affecting amino acid positions highly conserved across species (Supplementary Fig. S1). Although the frequency of these variants is higher among the PD exomes investigated than that in European samples from ExAC database ( $p = 0.075466$  and  $0.109979$  after Fisher exact test) we think that the data presented here are not sufficient to definitively support their role in the etiology of PD and that further evaluation in larger cohorts of PD patients is needed.

In an effort to understand the cumulative effect of rare variants (MAF  $\leq$  0.01) in this gene on the risk of PD, a set of SKAT-O tests were performed in this same data set, considering different levels of variant functionality (Supplementary Methods). None of these tests derived significant results (lowest  $p = 0.34$  when considering coding variants only, Supplementary Table S2).

Because the main finding described by Deng et al. derives from the analysis of a large family with autosomal dominant PD, we also investigated whether mutations in *TMEM230* cosegregated with this disease in WES/whole genome sequencing data from 29 multiplex dominant families from different European populations. In a large PD family we previously reported (Kindred A) (Nicholl et al., 2002), we found a rare variant in 2 cousins. This variant (rs374122606; NM\_001009923:c.G487A:p.A163T) has only 2 alleles in European populations from ExAC (MAF = 0.00001647) and was predicted damaging (Table 1). However, linkage analysis suggested

**Table 1**  
Variants of interest identified in the databases investigated

CHR	Position	Gene	Transcript ID: cDNA change: aminoacid change	ExACDBaltAF	CADD_phred
20	5081505	<i>TMEM230</i>	NM_001009923: c.A484G: p.I162V	0.000116279	22.2
20	5086929	<i>TMEM230</i>	NM_001009923: c.T316C: p.Y106H	0.000008132	24
20	5081502	<i>TMEM230</i>	NM_001009923: c.G487A: p.A163T	0.000016470	34

Variants identified in the samples investigated.

Key: CADD\_phred, phred-scaled CADD score for each variant (<http://cadd.gs.washington.edu/>); cDNA, complementary DNA; CHR, chromosome; ExACDBaltAF, alternative allele frequency in ExAC database (<http://exac.broadinstitute.org/>).

that this region is unlikely to be harboring the gene that causes PD in this family (Supplementary Fig. S2). A polymerase chain reaction followed by Sanger sequencing (primers available upon request) confirmed that the identified variant does not segregate with PD in this family.

In summary, our results do not support a genetic role of *TMEM230* both in the general Caucasian population and a set of families with autosomal dominant PD. Although we identified 2 variants in 2 PD cases that are not present in our control database, lack of family history, as well as the presence of the identified variants in ExAC database, makes it difficult to understand whether they represent pathogenic mutations or very rare benign events. We also identified a pathogenic variant in 2 PD cases from the same family. However, linkage data suggest that this variant is not the cause of PD in this family. A series of SKAT-O tests also failed to show a cumulative effect of rare variation in this gene on the risk of PD in the general Caucasian population. Further evaluation of whole exome/genome sequencing data from the PD research community is needed to clarify the role of this gene in the general population.

#### Disclosure statement

The authors declare they have no conflicts of interest, financial, or otherwise, related to the present work.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2016.10.004>.

#### References

- Amendola, L.M., Dorschner, M.O., Robertson, P.D., Salama, J.S., Hart, R., Shirts, B.H., Murray, M.L., Tokita, M.J., Gallego, C.J., Kim, D.S., Bennett, J.T., Crosslin, D.R., Ranchalis, J., Jones, K.L., Rosenthal, E.A., Jarvik, E.R., Itsara, A., Turner, E.H., Herman, D.S., Schleit, J., Burt, A., Jamal, S.M., Abrudan, J.L., Johnson, A.D., Conlin, L.K., Dulik, M.C., Santani, A., Metterville, D.R., Kelly, M., Foreman, A.K., Lee, K., Taylor, K.D., Guo, X., Crooks, K., Kiedrowski, L.A., Raffel, L.J., Gordon, O., Machini, K., Desnick, R.J., Biesecker, L.G., Lubitz, S.A., Mulchandani, S., Cooper, G.M., Joffe, S., Richards, C.S., Yang, Y., Rotter, J.I., Rich, S.S., O'Donnell, C.J., Berg, J.S., Spinner, N.B., Evans, J.P., Fullerton, S.M., Leppig, K.A., Bennett, R.L., Bird, T., Sybert, V.P., Grady, W.M., Tabor, H.K., Kim, J.H., Bamshad, M.J., Wilfond, B., Motulsky, A.G., Scott, C.R., Pritchard, C.C., Walsh, T.D., Burke, W., Raskind, W.H., Byers, P., Hisama, F.M., Rehm, H., Nickerson, D.A., Jarvik, G.P., 2015. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Res.* 25, 305–315.
- Deng, H.X., Shi, Y., Yang, Y., Ahmeti, K.B., Miller, N., Huang, C., Cheng, L., Zhai, H., Deng, S., Nuytemans, K., Corbett, N.J., Kim, M.J., Deng, H., Tang, B., Yang, Z., Xu, Y., Chan, P., Huang, B., Gao, X.P., Song, Z., Liu, Z., Fecto, F., Siddique, N., Foroud, T., Jankovic, J., Ghetti, B., Nicholson, D.A., Krainc, D., Melen, O., Vance, J.M., Pericak-Vance, M.A., Ma, Y.C., Rajput, A.H., Siddique, T., 2016. Identification of *TMEM230* mutations in familial Parkinson's disease. *Nat. Genet.* 48, 733–739.
- Hofman, A., Brusselle, G.G., Darwish Murad, S., van Duijn, C.M., Franco, O.H., Goedegebure, A., Ikram, M.A., Klaver, C.C., Nijsten, T.E., Peeters, R.P., Stricker, B.H., Tiemeier, H.W., Uitterlinden, A.G., Vernooij, M.W., 2015. The Rotterdam Study: 2016 objectives and design update. *Eur. J. Epidemiol.* 30, 661–708.
- Hofman, A., Darwish Murad, S., van Duijn, C.M., Franco, O.H., Goedegebure, A., Ikram, M.A., Klaver, C.C., Nijsten, T.E., Peeters, R.P., Stricker, B.H., Tiemeier, H.W., Uitterlinden, A.G., Vernooij, M.W., 2013. The Rotterdam Study: 2014 objectives and design update. *Eur. J. Epidemiol.* 28, 889–926.
- Ikram, M.A., van der Lugt, A., Niessen, W.J., Krestin, G.P., Koudstaal, P.J., Hofman, A., Breteler, M.M., Vernooij, M.W., 2011. The Rotterdam scan study: design and update up to 2012. *Eur. J. Epidemiol.* 26, 811–824.
- Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., Shendure, J., 2014. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat. Genet.* 46, 310–315.
- Lee, S., Emond, M.J., Bamshad, M.J., Barnes, K.C., Rieder, M.J., Nickerson, D.A., Team, N.G.E.S.P.-E.L.P., Christiani, D.C., Wurfel, M.M., Lin, X., 2012. Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. *Am. J. Hum. Genet.* 91, 224–237.
- Nicholl, D.J., Vaughan, J.R., Khan, N.L., Ho, S.L., Aldous, D.E., Lincoln, S., Farrer, M., Gayton, J.D., Davis, M.B., Piccini, P., Daniel, S.E., Lennox, G.G., Brooks, D.J., Williams, A.C., Wood, N.W., 2002. Two large British kindreds with familial Parkinson's disease: a clinico-pathological and genetic study. *Brain* 125 (Pt 1), 44–57.