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LETTER TO THE EDITOR

No evidence for rare *TRAP1* mutations influencing the risk of idiopathic Parkinson's disease

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Sir,

In their recent work, Fitzgerald *et al.* (2017) report a novel homozygous *TRAP1* loss-of-function mutation in a patient with late-onset Parkinson's disease. Further, they show an enrichment of two subgroups of rare *TRAP1* variants in controls compared to patients with Parkinson's disease in the Parkinson's Progression Markers Initiative (PPMI) dataset (Parkinson Progression Marker Initiative, 2011). However, these associations are not significant after correction for multiple testing. The enrichment is measured using the burden and SKAT-O (Lee *et al.*, 2012) tests. From this, the authors stipulate that rare, more benign missense *TRAP1* mutations are depleted in patients with Parkinson's disease.

Here, we sought to replicate these findings and investigate the role of *TRAP1* mutations in our exome sequencing dataset, comprising 181 Parkinson's disease cases from the Norwegian ParkWest cohort (Alves *et al.*, 2009) and 196 in-house controls (unpublished results). Following quality control, variants were annotated using ANNOVAR (Wang *et al.*, 2010) according to the RefSeq gene transcripts, dbNSFP v3.3a (Liu *et al.*, 2016) and ExAC (Lek *et al.*, 2016). We identified 21 exonic variants in the *TRAP1* gene, of which 16 were non-synonymous (missense) and five were synonymous. We did not detect the specific p.R47X mutation described by Fitzgerald *et al.*, nor did we find any other nonsense or splice mutations. Two missense variants were present in cases only (in heterozygous form), but they were predominantly predicted to be benign/tolerated across five different prediction algorithms (SIFT, PolyPhen-2 HumVar/HumDiv, LRT and MutationTaster). No single variant association test was significant after correction for multiple testing.

For collapsing tests, we selected variants with minor allele frequency (MAF) < 1% in the non-Finnish European ExAC dataset. We created subsets of variants within *TRAP1* based on synonymy and CADD score similarly to Fitzgerald *et al.* In addition to burden and SKAT-O, we also performed the

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Table 1 Region-based analysis of TRAP1 variants

Group	Number of variants	MAC controls	MAC cases	Burden *P*-value	SKAT-O *P*-value	SKAT *P*-value
The Norwegian ParkWest sample						
Non-synonymous	12	18	12	0.407	0.648	0.566
CADD10	11	14	7	0.130	0.221	0.806
CADD15	9	7	4	0.229	0.379	0.751
CADD20	9	7	4	0.229	0.379	0.751
CADD30	2	2	0	0.326	0.489	0.786
Synonymous	2	2	0	0.332	0.746	0.746
The PPMI sample						
Non-synonymous	9	5	7	0.279	0.367	0.255
CADD10	8	5	6	0.259	0.382	0.205
CADD15	8	5	6	0.259	0.382	0.205
CADD20	6	4	5	0.293	0.312	0.259
CADD30	2	1	1	0.799	0.277	0.277
Synonymous	2	0	2	0.338	0.739	0.739

CADD = non-synonymous variants with CADD score > 10, 15, 20 and 30, respectively.
MAC = minor allele count.
P-values are uncorrected for multiple testing.

SKAT test (Wu *et al.*, 2011). Collapsing tests were performed using the SKAT R package (Lee *et al.*, 2016). We found no evidence of variant enrichment in *TRAP1*, in any of the tests/models tested in our population. The results are summarized in Table 1.

Upon close examination of the analyses performed by Fitzgerald *et al.* in the PPMI cohort, we raise a few questions regarding aspects of the quality control and collapsing testing. Firstly, the authors use a particularly lax threshold for variant call-rate ($\geqslant$90%). Missing genotypes may be due to genotyping errors, and region-based collapsing tests using rare variants are particularly susceptible to inflated type I error rates if the distribution of missed calls differs between cases and controls in a tested region (Auer *et al.*, 2013). Another crucial aspect when testing for rare variant associations is the control of population stratification. Rare variants display very little sharing between populations (Gravel *et al.*, 2011), and failure to control for this could therefore lead to spurious associations, especially in a heterogeneous sample such as the PPMI. While removing individuals 3 standard deviations (SD) from the mean of the first and second principal component does reduce ethnic heterogeneousness to some degree, a more prudent approach would perhaps have been to remove outliers iteratively, as implemented by Eigensoft (Patterson *et al.*, 2006; Price *et al.*, 2006).

Considering the above limitations, we sought to replicate the findings of the study in the same PPMI dataset, but following a more stringent quality control procedure. Specifically, we used a variant call-rate cut-off of >98% and performed principal component analysis using Eigensoft with standard filtering settings (five iterations, 10 principal components, sigma 6), in addition to removing outliers ($\geqslant$3 SD) across the first and second principal components post-filtering. Rare variants were defined as variants with MAF < 0.5% in the non-Finnish European ExAC dataset to replicate the parameters described by Fitzgerald *et al.* In this robustly quality controlled dataset, we detected no nominally significant variant enrichment in *TRAP1* by either burden, SKAT-O or SKAT tests. The results of our replicative PPMI analyses are summarized in Table 1.

In conclusion, while the reported p.R47X *TRAP1* mutation may indeed be deleterious to mitochondrial function, no definite evidence is provided that this mutation is the cause of Parkinson's disease in the reported case. Moreover, we found no evidence supporting that rare variation enrichment in *TRAP1* influences the risk of Parkinson's disease in two independent populations. We therefore believe that the proposed role of *TRAP1* in Parkinson's disease is unsubstantiated by the data presented in the study.

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