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181 Parkinson’s disease cases from the Norwegian ParkWest cohort (Alves et al., 2009) and 196 in-house controls (unpublished results).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. 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
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 ($\geq 90\%$). 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 heterogeneity 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 $\geq 98\%$ and performed principal component analysis using Eigensoft with standard filtering settings (five iterations, 10 principal components, sigma 6), in addition to removing outliers ($\geq 3$ 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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**Table 1 Region-based analysis of TRAP1 variants**

| Group                  | Number of variants | Number of variants | Number of variants | Number of variants | 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.

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