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LETTER TO THE EDITOR

ARSA variants in α-synucleinopathies

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Sir,

We read with great interest the recently published article by Lee and colleagues reporting variants in *ARSA* and their association with Parkinson's disease (Lee *et al.*, 2019). Deficiency of arylsulfatase A is a known cause of metachromatic leukodystrophy (MLD), an autosomal recessive lysosomal storage disease. The study describes a patient with MLD and a family history of Parkinson's disease. The patient was a compound heterozygous carrier of two rare missense *ARSA* mutations, p.L300S (c.899T>C, rs199476389) and p.C174Y (c.521G>A, rs199476381). Screening of *ARSA* in two family members with Parkinson's disease and two unaffected members found that the p.L300S mutation segregated with Parkinson's disease, but not the p.C174Y mutation. Next, a candidate gene analysis of *ARSA* was conducted in 92 familial and 92 sporadic Parkinson's disease patients, and the results were compared to the allele frequencies within the Integrative Japanese Genome Variation Database. This screening identified a common missense variant, p.N352S (c.1055A>G, rs2071421), that was more frequent in healthy Japanese individuals than in familial and sporadic Parkinson's disease cases (P = 0.026 and P = 0.0349, respectively). The authors concluded that the p.N352S variant may be protective against the development of Parkinson's disease. They also found that *ARSA* deficiency increases α-synuclein aggregation and secretion, suggesting a potential link between *ARSA* mutations and α-synuclein pathology.

α-Synucleinopathies are a heterogeneous group of neurodegenerative disorders characterized by fibrillar aggregates of insoluble α-synuclein protein in the cytoplasm of specific neurons and glial cells. These disorders include Parkinson's disease, Lewy body dementia (LBD), multiple system atrophy (MSA), and REM-sleep behaviour disorder (RBD), a prodromal α-synucleinopathy (Goedert *et al.*, 2017; Postuma *et al.*, 2019). Advances in genetics have implicated lysosomal dysfunction in the pathogenesis of several α-synucleinopathies. For example, variants within the

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lysosomal genes *GBA* (Sidransky *et al.*, 2009) and *SMPD1* (Alcalay *et al.*, 2019) have been associated with an increased risk of Parkinson's disease. Accumulation of α-synuclein has been observed in some lysosomal storage disorders suggesting a pathobiological link between these two disease groups (Shachar *et al.*, 2011). Similarly, *GBA* variants have been associated with LBD (Nalls *et al.*, 2013; Geiger *et al.*, 2016), MSA (Mitsui *et al.*, 2015; Sklerov *et al.*, 2017) and RBD (Gan-Or *et al.*, 2015). These intriguing observations prompted us to investigate *ARSA* variants in cohorts of α-synucleinopathies.

First, we sought to examine the association between the *ARSA* p.N325S variant and α-synucleinopathies by analysing genome-wide association study (GWAS) data from cohorts of Parkinson's disease cases and proxy cases (n = 56 306 cases, n = 1 417 791 controls), LBD (n = 556 cases, n = 1418 controls), MSA (n = 896 cases, n = 3881 controls) and RBD (n = 1046 cases, recruited as isolated, polysomnography-confirmed RBD before conversion to α-synucleinopathy, n = 11 961 controls). All participants were of European ancestry and underwent similar genotyping, and standardized quality control procedures are described in detail elsewhere (Sailer *et al.*, 2016; Nalls *et al.*, 2019). The common p.N352S variant was reliably imputed in all cohorts ($R^2 > 0.9$; allele frequency distributions are shown in Table 1). In the Parkinson's disease cohort, the allele frequencies of the p.N325S variant were very similar in patients (0.1334) and controls (0.1354). In other α-synucleinopathy cohorts, the direction of effect was not consistent (Table 1). After correction for multiple testing, our analyses found no significant association of the p.N352S *ARSA* variant with α-synucleinopathies.

Next, we aimed to examine whether rare, potentially pathogenic variants in *ARSA* are associated with α-synucleinopathies. For this purpose, we performed burden analysis of these *ARSA* variants (annotated as stop-gain, frameshift, or marked as 'pathogenic' by ClinVar) in European-ancestry exome datasets from 1311 Parkinson's disease patients and 571 matched control subjects, demonstrating lack of association, with higher frequency of potentially pathogenic variants in controls (frequency in patients/controls = 0.0015/0.004, P = 0.226). We further performed burden analysis in 264 definite MSA patients and 462 neuropathologically healthy control subjects (Pihlstrom *et al.*, 2018) (including non-synonymous variants only, no frameshift, stop-gain or ClinVar 'pathogenic' variants were identified in this cohort), and here too, no association was found (frequency in patients/controls = 0.0076/0.0043, P = 0.517). The p.L300S variant was not observed in any of these datasets.

We are concerned about several conclusions regarding the p.N352S variant and the role of pathogenic *ARSA* variants that have been drawn in the Lee *et al.* (2019) article. First, as p.N352S is a common polymorphism (Table 1), a GWAS would be able to determine with certainty if this locus is significant on a genome-wide level, and this is not seen in well-powered cohorts, including a Japanese Parkinson's disease GWAS of 2011 patients and 18 381 controls, which did not identify an association in this locus (Satake *et al.*, 2009). Second, the hypothesis arguing that p.N352S is protective in autosomal dominant Parkinson's disease would ideally be investigated by assessing penetrance or age at onset in carriers of known autosomal dominant variants. Third, regarding p.N352S being a coding and reportedly functional variant, population-specific effects are unlikely. The variant shows particularly variable frequencies across populations in gnomAD, with frequencies between 0.06 and 0.33 in different populations (0.1243 and 0.1733 in European and East Asian populations, respectively, https://gnomad.broadinstitute.org/). Given the high frequency of the variant, it is unlikely that it has a large effect size. The authors nominated their protective variant based on a comparison between a small cohort of sporadic Parkinson's disease patients (n = 92) and a Japanese database, seemingly without adjustment for covariates, such as age, sex, or ancestry. In addition, the same healthy individuals were used for comparing both the familial and sporadic Parkinson's disease cohorts. Consequently, bias within this small control cohort may have affected the results. Lastly, our burden analyses, as well as a previous burden analysis (Robak *et al.*, 2017), did not identify an association between rare, potentially pathogenic *ARSA* variants and α-synucleinopathies.

In conclusion, our analyses do not support a significant association between common and rare *ARSA* variants and

Table 1 Analysis of the p.N352S *ARSA* variant in α-synucleinopathies

Cohort	Cases, *n*	Controls, *n*	Frequency (affected)	Frequency (unaffected)	*P*-value[a]	OR (CI)
LBD GWAS[b]	556	1418	0.1061	0.1326	0.024	0.77 (0.623–0.967)
MSA GWAS[b]	896	3881	0.1384	0.1336	0.592	1.042 (0.897–1.209)
RBD GWAS[b]	1046	11 961	0.1456	0.1292	0.030	1.16 (1.015–1.335)
PD GWAS[b]	56 306[c]	1 417 791	0.1334[d]	0.1354[d]	0.022	0.969 (0.943–0.996)

CI = confidence interval; GWAS = genome-wide association study; LBD = Lewy body dementia; MSA = multiple system atrophy; OR = odds ratio; PD = Parkinson's disease; RBD = REM sleep behaviour disorder.

[a]Uncorrected *P*-value, all results were not significant after correction for multiple comparisons.

[b]p.N352S was found in imputed genotyping files with an R^2 value of 0.975 for dementia with Lewy bodies GWAS, 0.953 for multiple system atrophy GWAS, 0.997 for RBD GWAS, and >0.96 for Parkinson's disease GWAS.

[c]Including proxy cases.

[d]Frequency estimates were based on a subset of the data including 21 478 cases and 24 388 controls.

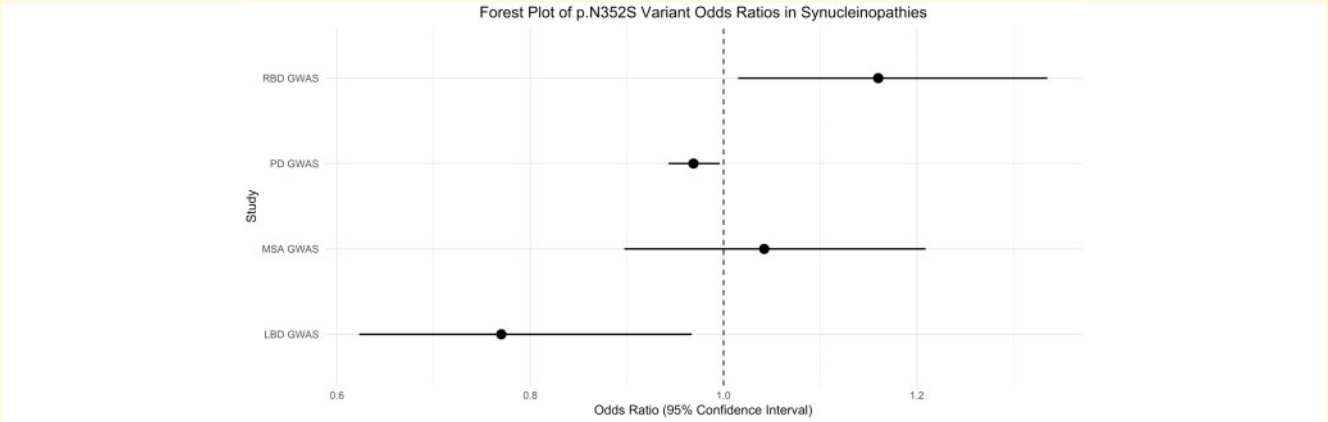

Figure 1 Forest plot of the p.N352S variant odds ratios and 95% confidence intervals in α-synucleinopathies.

α-synucleinopathies despite adequate power. Our cohorts were of European ancestry and it is possible that with a larger Asian cohort, the reported association of the *ARSA* p.N352S variant with Parkinson's disease would be lost and mimic our findings. However, our results do not completely rule out a potential role for *ARSA* in Parkinson's disease, and additional large-scale familial and case-control studies are necessary to determine whether *ARSA* is associated with α-synucleinopathies.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Competing interests

The authors report no competing interests.

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