Intronic haplotypes in GBA modify age at diagnosis of Parkinson's: replication in a subgroup

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Intronic Haplotypes in GBA Modify Age at Diagnosis of Parkinson's: Replication in a Subgroup

In Schierding et al we identified noncoding variants within *GBA* that were associated with age at PD onset and diagnosis.[1] Toffoli et al (this issue) failed to replicate our findings using data from the RAPSODI study and AMP-PD cohort. Here we provide evidence that supports our original findings and discuss the hypothesis that differing diagnostic criteria and/or data conglomeration is a potential basis for the replication failure of Toffoli et al.

Methods

The cohort and methods for polymerase chain reaction amplification and sequencing the *GBA* gene, and not the the pseudogene *GBAP1*, were previously described.[2] For this analysis, patients were classified according to referring neurologist (Fig. 1).

Results

Haplotyping analysis of the Netherlands cohort of 1242 patients lacking *GBA* exonic variants did not replicate our findings (Fig. 1A, All). However, stratification by referral source identified a significant association (P = 0.0022) between the *GBA1* intronic haplotype and age at diagnosis (AAD) in individuals who were referred to the study by tertiary center–based neurologists (Fig. 1A, Tertiary). The difference between the median ageat diagnosis for the AA and BB *GBA1* intronic haplotypes was 10 years with weak evidence for a dosage effect (Fig. 1B). This finding was consistent with our original observation of a dosage effect and 3.4-year median difference in age at diagnosis observed between the 208 deeply phenotyped PD patients (AA vs BB) in the NZBRI cohort, who were diagnosed by a single clinician at a movement disorders clinic.[1,3]

We observed the identification of a significant haplotype–AAD relationship within the tertiary-diagnosed patients and not those from the other categories (peripheral, mix, and other(mix); Fig. 1). This observation may suggest that populations of patients who are at tertiary clinics are distinct from other populations. There are at least 2 nonexclusive explanations for this. First, it could reflect a scenario in which the diagnostic process for PD, and consequently AAD, varies between cohorts. If so, amalgamating patients diagnosed using differing diagnostic processes into a cohort is likely to obscure potential haplotype–AAD associations. As such, the observation that the RAPSODI study and the multiple cohorts that make up AMP-PD use differing diagnostic criteria is a concern (Supplementary Table 1). Data conglomeration issues like these are a recognized confounder for genomic studies because of variability in the phenotyping.[4] Alternatively, it could be argued that some subtypes (eg, early onset or high familial burden) of PD patients are preferentially referred to and examined by tertiary neurologists. This could lead to the tertiary cohort having specific characteristics that are associated with the observed genetic trend.

Alternative explanations for our observations also include: (1) the sample sizes of the NZBRI and Netherlands PD (tertiary) are not sufficiently large, and the association is a false positive; (2) founder effects are present in both the NZBRI and Netherlands PD cohorts.

Finally, it is possible that the haplotype–AAD association was not detected in the AMP-PD because the accurate mapping of short-sequencing reads to GBA in AMP-PD is confounded by reads from the highly similar *GBAP1* pseudogene. By contrast, the NZBRI and Netherlands cohorts underwent targeting sequencing of *GBA*.

Large cohorts with harmonized clinical, genomic, and transcriptomic datasets are critical resources for the breakthrough discoveries required to substantially advance our understanding of disease, its different trajectories, and the identification of potential therapeutic targets. However, as this study has indicated, potential variation in phenotyping, either within a cohort or between cohorts, has the capacity to diminish evidence of possibly important findings.

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Data Availability Statement

Data is available from Heijer et al.

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Key Words: Parkinson's; *GBA*; intronic variants; age at onset; age at diagnosis

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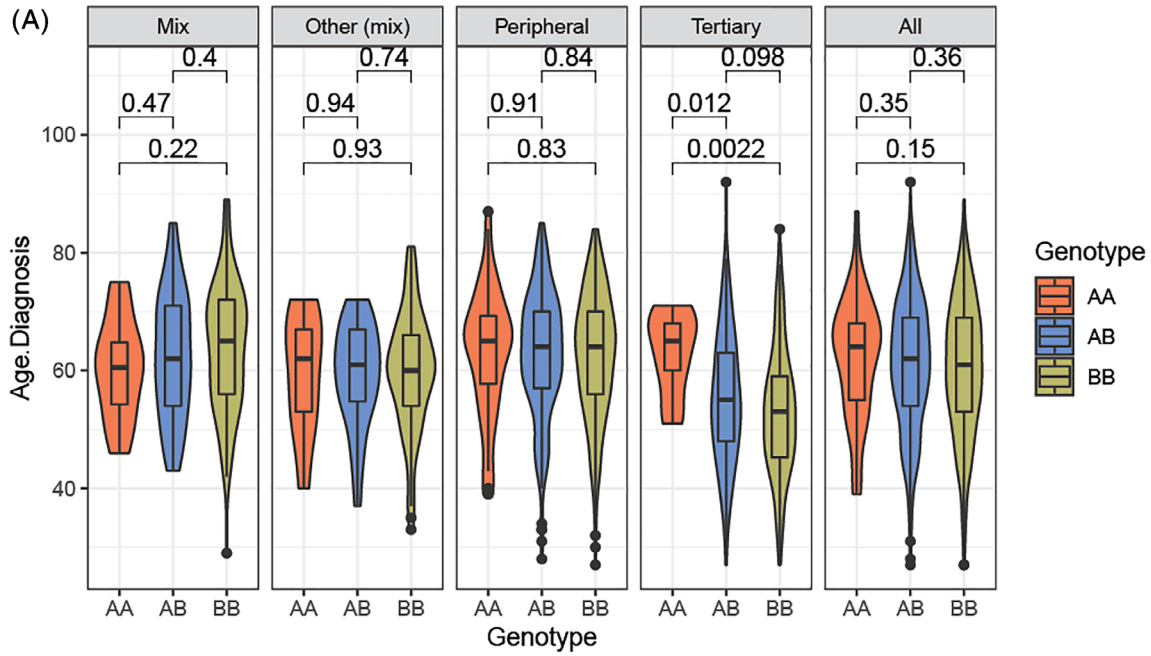

(B)

			Age at diagnosis	
Hospital type	Genotype	Number	Mean +/- SD	Median
Mix	AA	12	60.3 ± 8.7	60.5
Mix	AB	77	62.4 ± 10.6	62
Mix	BB	93	63.8 ± 11	65
Other (mix)	AA	9	59.4 ± 10.4	62
Other (mix)	AB	36	59.2 ± 8.8	61
Other (mix)	BB	81	59.8 ± 10.1	60
Peripheral	AA	48	62.9 ± 11	65
Peripheral	AB	281	62.7 ± 10.6	64
Peripheral	BB	358	62.6 ± 10.6	64
Tertiary	AA	9	63 ± 7	65
Tertiary	AB	96	55.4 ± 11.3	55
Tertiary	BB	142	52.9 ± 10.8	53
All	AA	78	62.1 ± 10.1	64
All	AB	490	61.0 ± 11.0	62
All	BB	674	60.4 ± 11.3	61

FIG. 1. Conglomeration of wild-type (no exonic mutations) *GBA1* sequencing data across diagnostic cohorts obscures the relationship between the intronic *GBA* haplotype and age of onset. (**A**) Violin plot illustrating the association of *GBA1* intronic haplotype (AA, homozygous Ref allele (T/T T/T G/G); AB, heterozygous; BB, homozygous Alt allele (G/G C/C A/A) with age of diagnosis. Patients were classified according to where their neurologist was based. Mix, referral was by a combination of both university- and nonuniversity-based neurologists from the northern Netherlands; Peripheral, referral by a neurologist in a non-university center; Tertiary, referral by a neurologist in a tertiary university center; Other(mix), self-referral to the study based on a neurologist diagnosis from a combination of university and nonuniversity centers; ALL, all patients in the study. Statistical significance was tested using the Student *t* test and results plotted using R-Shiny. (**B**) Summary data for each category in (**A**). [Color figure can be viewed at wileyonlinelibrary.com]

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Chronic Immunosuppression and Potential Infection Risks in *CSF1R*-Related Leukoencephalopathy

We have read the viewpoint article by Tipton and colleagues titled "Is Pre-Symptomatic Immunosuppression Protective in *CSF1R*-Related Leukoencephalopathy?" with great interest.[1] In this article the authors described an interesting case carrying a *CSF1R* gene mutation coexisting with rheumatoid arthritis who had been treated with various immunosuppressive drugs including prednisone, hydroxychloroquine, methotrexate, and adalimumab for 25 years. The 71-year-old woman did not exhibit typical clinical and radiological features of leukoencephalopathy, while her daughter was clinically diagnosed with *CSF1R*-related leukoencephalopathy at the age of 43 years.[1] In both individuals genetic testing revealed the same mutation in the *CSF1R* gene.

CSF1R-related leukoencephalopathy is a severe neurodegenerative disease with microglia in the central nervous system playing a crucial role.[2] Decreased numbers of microglia with significant morphological alterations are evident in human brains of *CSF1R*-related leukoencephalopathy.[3] It is widely recognized that microglia provide the first line of defense against invading pathogens. One previous preclinical study has demonstrated that depletion of microglia using the CSF1R inhibitor PLX5622 could lead to delayed clearance of neurotropic coronavirus and increased viral replication,[4] suggesting that microglia play a key role in host protection from viral infection. Our colleagues have recently shown that patients with multiple sclerosis, following highly effective disease-modifying drug treatment, have an increased risk of infections compared with the general population, with variation depending on the drug.[5] The concerns of potentially increased infection risks associated with dysfunctional innate immunity and chronic immunosuppression in *CSF1R*-related leukoencephalopathy should be considered during risk–benefit assessment, particularly in the current COVID-19 pandemic.

The case carrying a *CSF1R* gene mutation described by the authors is special because this patient also suffered from rheumatoid arthritis, a disease state that is associated with various immune cells involved in disease pathogenesis. In addition, it is important to point out that previously reported *CSF1R*-related leukoencephalopathy individuals after disease onset did not show significant clinical improvement following treatment with glucocorticoids.[6,7] One patient received a single methylprednisolone pulse therapy (1000 mg/day for 5 days),[7] while another case received an unknown dose and treatment duration.[6] Further characterization of the treatment duration and dose, the time of treatment start, and standardized functional scales is therefore needed to evaluate the efficacy and safety of long-term immunosuppression in patients with *CSF1R*-related leukoencephalopathy.

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Key Words: *CSF1R* gene; adult-onset leukoencephalopathy with spheroids and pigmented glia; immunosuppression; glucocorticoids; infection

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