Genetic and clinical pharmacology studies in GBA1-associated Parkinson's disease

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CHAPTER 5

Intronic haplotypes in *GBA* modify age at diagnosis of Parkinson's: replication in a sub-group

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Introduction

In Schierding et al. we identified non-coding variants within *GBA* that were associated with age of PD onset and diagnosis1. 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 PCR amplification and sequencing the *GBA* gene, and not the the pseudogene *GBA*P1, were previously described.[2] For this analysis, patients were classified according to referring neurologist (Figure 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 centre-based neurologists (Fig. 1A, Tertiary). The difference between the median age-of-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-of-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 two non-exclusive explanations for this. Firstly, 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 comprise AMP-PD use differing diagnostic criteria is a concern (Supplementary Table 1). Data conglomeration issues like these are a recognized confounder for genomic studies due to variability in the phenotyping4. Alternatively, it could be argued that some sub-types (e.g. 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 samples 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 *GBA*P1 pseudogene. By contrast, the NZBRI and Netherlands cohorts underwent targeting sequencing of *GBA*.

Large cohorts with harmonised 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.

REFERENCES

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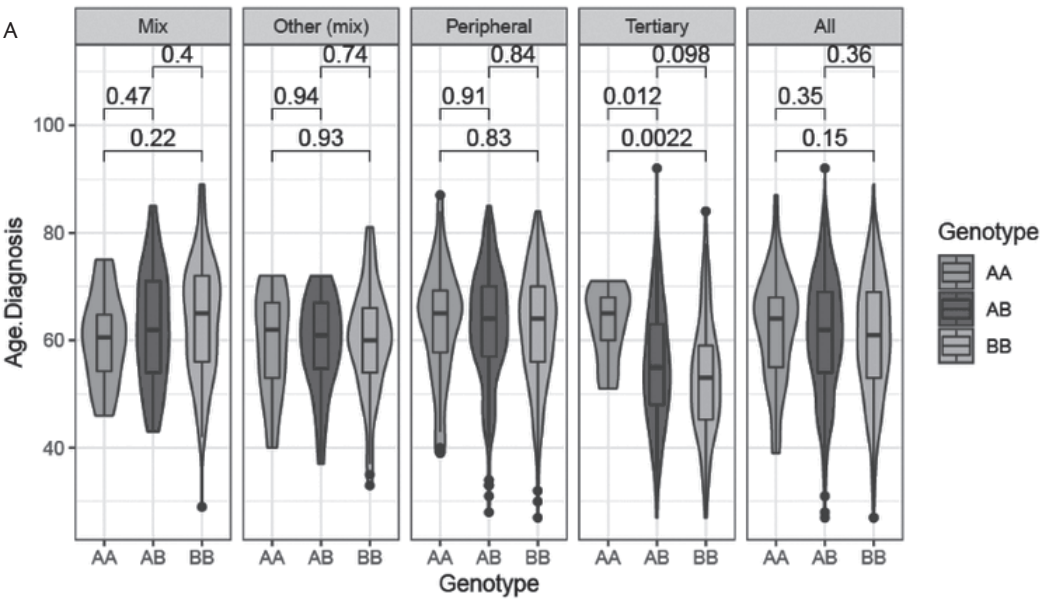
Figure 1 Figure 1. Conglomeration of wild-type (no exonic mutations) *GBA1* sequencing data across diagnostic cohorts obscures the relationship between intronic *GBA* haplotype and age of onset. A) Violin plot illustrating the association of *GBA1* intronic haplotype (AA – homozygous Ref allele (T/T 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 non-university based neurologists from the northern Netherlands; Peripheral, referral by a neurologist in a non-university center; Tertiary, referral by a neurologist located in a tertiary university center; Other(mix), self-referral to the study based on a neurologist diagnosis from a combination of university and non-university centers; ALL, all patients in the study. Statistical significance was tested using the Students t-test and results plotted using R-Shiny. B) Summary data for each category in A).

A



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

SCAN ME

SUPPLEMENTARY MATERIAL

H5ST1