GBA variants influence cognitive status in amyotrophic lateral sclerosis

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive degenerative disease of upper and lower motor neurons. Approximately 15% of patients display clinical features consistent with frontotemporal dementia (FTD) and 35% display milder degrees of cognitive and behavioural impairment at some stage during their illness. Several genes have been reported to cause both ALS and FTD. Nevertheless, it remains unclear why some patients with ALS develop cognitive impairment, while other cases, often within the same family, remain unaffected.

The GBA gene (OMIM *606463) encodes glucocerebrosidase (GCase), a lysosomal enzyme that converts glucocerebroside into glucose and ceramide. Heterozygous GBA mutations increase the risk of Parkinson’s disease (PD) and the risk of cognitive impairment in patients with PD.1

It is increasingly recognised that variants in genes causing Mendelian neurodegenerative diseases may exhibit pleiotropic effects and impact the phenotypic heterogeneity of those disorders. Moreover, lysosomal dysfunction has recently been associated with both Dementia with Lewy Bodies and FTD spectrum (online supplemental table 1). Based on this, we postulated that GBA variants may influence the cognitive status of patients with ALS.

MATERIALS AND METHODS

We examined the GBA variants’ association with the risk of cognitive impairment in 751 patients with ALS from the population-based Piemonte and Valle d’Aosta Register for ALS that had undergone both a detailed neuropsychological evaluation and a whole-genome sequencing screening.1 Patients were classified as ALS with normal cognitive function (ALS-CN), ALS-FTD and ALS with intermediate cognitive deficits. The characteristics of the study population and a detailed description of neuropsychological testing and genetic screening are reported in online supplemental materials in the Methods section.

A mutational screening of GBA exonic variants was performed and their frequencies were compared with an internal control cohort (see online supplemental methods, Subjects). To assess whether pathogenic rare variants (minor allele frequency<1%) in GBA contribute to cognitive decline risk in ALS, a gene-based rare variants association test was performed as previously described.1 In the following step of the analysis, only variants known to be a risk factor for cognitive decline in PD were considered. First, a binomial test was used to assess the prevalence of GBA mutations across cognitive groups. Then, a linear mixed-effects model was used to test for associations between GBA genotype and cognitive functioning while including the following covariates: sex, age, site of disease onset, bulbar signs at diagnosis, rate of ALS Functional Rating Scale-Revised (ALS-FRS-R) decline and C9orf72 status.

We identified one GBA mutation (p.N409S), known to cause Gaucher Disease in homozygous carriers, one likely pathogenic variant (p.R209H) and two GBA polymorphisms that are known to increase the risk of dementia in patients with PD (p.E365K and p.T408M). The remaining identified coding GBA variants are reported in online supplemental table 2. The frequency of GBA variants was not increased in our cohort as compared with healthy controls (online supplemental table 4). Thirteen out of 18 (72.2%) of patients with ALS carrying GBA variants displayed cognitive impairment in the form of FTD or intermediate cognitive phenotypes. In contrast, cognitive impairment was observed among 47.1% (298 out of 733) of patients with ALS not carrying GBA variants (binomial test p value=0.0357). We repeated the analysis excluding C9orf72 expansion carriers and the difference remained significant (p=0.0486). To confirm the effect of GBA variants on cognitive phenotype, we modelled the association between the GBA variants and cognitive impairment using a linear mixed-effects model that controlled for relevant covariates (figure 1).

RESULTS

The gene-based rare variant association test identified an enrichment of rare GBA variants in patients with ALS with intermediate cognitive dysfunction (p SKAT-O=0.000005), but not in ALS-FTD cases (p SKAT-O=0.184) (online supplemental table 2).

GBA variants represent a detrimental factor. Our possible role of GBA in the neurodegenerative process underlying ALS is suggested by increasing evidence of the involvement of endolysosomal dysfunction in ALS pathogenesis. Several genes causing ALS and FTD, including C9orf72, TBK1, OPTN, SQSTM1 and VCP, are related to lysosomal function and protein degradation. This research field deserves further attention as several therapeutic agents targeting lysosomal pathways have been proposed for neurological diseases.5 Our results expand the spectrum of neurodegenerative diseases for which heterozygous GBA variants represent a detrimental factor. It is possible that such variants are kept in populations because they provide some biological advantage.

As a limitation of our study, we acknowledge that we could not evaluate whether different variants had a variable impact on the risk of cognitive impairment, on the pattern of cognitive deficits and on other clinical characteristics, mostly due to the relatively small number of GBA variant carriers (online supplemental tables 5 and 6).

DISCUSSION

We observed that the burden of rare variants in the GBA gene was associated with cognitive impairment in patients with ALS. Patients carrying known pathogenic GBA variants (p.E365K, p.T408M, p.N409S) were three times more likely to develop cognitive impairment compared with non-carriers, independently of age, sex, site of onset, bulbar involvement, rate of ALS-FRS-R decline and C9orf72 status.

In multivariate analysis, we identified an effect of GBA variants only when ALS-CN cases were compared with patients with FTD and intermediate deficits combined. However, the results of the burden test suggest that this finding is primarily driven by patients with intermediate deficits. The course of cognitive deterioration among patients with ALS may partially explain our findings: recent studies have shown that cognitive impairment may worsen over time and that it is correlated to more severe motor deficits.3 The neuropsychological assessment at diagnosis might have captured an early phase of the trajectory of cognitive deterioration over time. Nonetheless, also the small number of GBA variant carriers may have conditioned such findings.

The gene-based rare variant association test identified an enrichment of rare GBA variants in patients with ALS with intermediate cognitive dysfunction (p SKAT-O=0.000005), but not in ALS-FTD cases (p SKAT-O=0.184) (online supplemental table 2).

REFERENCES

1. Patients were classified as ALS with normal cognitive function (ALS-CN), ALS-FTD and ALS with intermediate cognitive deficits. The characteristics of the study population and a detailed description of neuropsychological testing and genetic screening are reported in online supplemental materials in the Methods section.

2. It is increasingly recognised that variants in genes causing Mendelian neurodegenerative diseases may exhibit pleiotropic effects and impact the phenotypic heterogeneity of those disorders. Moreover, lysosomal dysfunction has recently been associated with both Dementia with Lewy Bodies and FTD spectrum (online supplemental table 1).

3. Thirteen out of 18 (72.2%) of patients with ALS carrying GBA variants displayed cognitive impairment in the form of FTD or intermediate cognitive phenotypes. In contrast, cognitive impairment was observed among 47.1% (298 out of 733) of patients with ALS not carrying GBA variants (binomial test p value=0.0357).

4. We repeated the analysis excluding C9orf72 expansion carriers and the difference remained significant (p=0.0486). To confirm the effect of GBA variants on cognitive phenotype, we modelled the association between the GBA variants and cognitive impairment using a linear mixed-effects model that controlled for relevant covariates (figure 1).

5. Our results expand the spectrum of neurodegenerative diseases for which heterozygous GBA variants represent a detrimental factor. It is possible that such variants are kept in populations because they provide some biological advantage.
In conclusion, we found that variants of the GBA gene are associated with an increased risk of cognitive impairment in patients with ALS. Our results broaden the spectrum of genetic factors that modulate the vulnerability of patients with ALS to cognitive dysfunction and strengthen the role oflysosomal impairment in the neurodegenerative process underlying ALS, highlighting that genes can modify not only the risk of ALS but also modulate different aspects of its phenotype. Addressing the gap in our understanding of the role that genetic modifiers play in ALS is essential for diagnosis, prognosis, and therapy development.

**Figure 1** (A) Cognitive phenotype frequencies in GBA risk variant carriers. Given the strong influence of C9orf72 on cognitive status, here we performed the binomial test without C9orf72 expansion carriers to rule out its impact on the results. (B) Results of the linear mixed-effects model, coefficient and 95% CI. Model estimates are expressed as e^{B0}. Colours correspond to the results of the three different models (1) ALS-CN versus ALS-FTD (blue); (2) ALS-CN versus GBA+ALS+ALS; (3) ALS-CN versus ALS-FTD + ALS-ALSci + ALS-ALS (green). See online supplemental file 1 for further information about cognitive classification. ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia.

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boards for Mitsubishi Tanabe, Roche, Denali Pharma, Cytokinetics, and Amylyx.

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REFERENCES