Letters

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.[1] 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.[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). 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.[3] 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 supplementarl 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.[3] 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. Further details on the statistical analysis are reported in online supplementary materials. All statistical analyses were performed in R V.3.6.0 (https://www.r-project.org/).

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).

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 3. 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). In the mixed-effects model, *GBA* mutation status was associated with the clinical diagnosis of cognitive impairment (OR=3.74, 95% CI 1.25 to 12.72, p=0.023). This effect was not seen when considering either only the ALS-FTD phenotype or the intermediate phenotype.

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.[4] 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.

A 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).

A

Phenotype	*GBA* risk variants carriers	*GBA* risk variants non carriers	Binomial test P-value (vs Cn)
ALS-FTD	4 (3.7%)	105 (96.3%)	0.1197
ALSbi/Ci/Cbi	8 (4.0%)	194 (96.0%)	0.0749
ALS-FTD + ALSbi/Ci/Cbi	12 (3.9%)	299 (96.1%)	0.0486
CN	5 (1.4%)	365 (98.6%)	
Total	17	664	

B

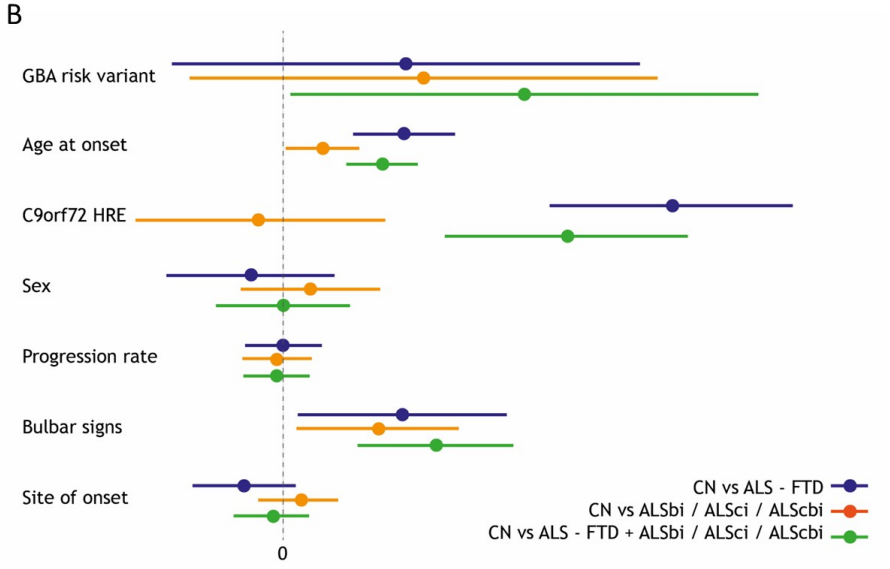

Figure 1 (A) Cognitive phenotype frequencies in *GBA* risk variant carriers. Given the strong influence of *C9orf72* on cognitive status, here we performed the binomial test without *C9orf72* expansion carriers to rule out its impact on the results. (B) Results of the linear mixed-effects model, coefficient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

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 of lysosomal 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.

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

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval The study was approved by the Ethical Committees of the two institutions involved in the study. Written informed consent was obtained at enrollment. The databases were anonymised according to Italian law for the protection of privacy.

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REFERENCES

1 Phukan J, Elamin M, Bede P, *et al*. The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. *J Neurol Neurosurg Psychiatry* 2012;83:102–8.
2 Sidransky E, Nalls MA, Aasly JO, *et al*. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med* 2009;361:1651–61.
3 Grassano M, Calvo A, Moglia C. Mutational analysis of known ALS genes in an Italian population-based cohort. *Neurology* 2020;96. doi:10.1212/WNL.0000000000011209
4 Beeldman E, Govaarts R, de Visser M, *et al*. Progression of cognitive and behavioural impairment in early amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2020;91:779–80.
5 Peng W, Minakaki G, Nguyen M, *et al*. Correction to: preserving lysosomal function in the aging brain: insights from neurodegeneration. *Neurotherapeutics* 2019;16:920–1.

Supplementary Methods

Subjects

The study population consisted of incident patients identified through the Piemonte and Valle d'Aosta Register for ALS (PARALS), diagnosed with definite, probable, and probable laboratory-supported ALS according to the El Escorial revised diagnostic criteria[1] between January 1st 2007 and December 31st 2015. The PARALS is a prospective epidemiologic registry established in 1995 in Piemonte and Valle d'Aosta regions in Northern Italy.[2] Patients were followed at the ALS Centers in Turin and Novara. Patients with a history of other neurologic disorders affecting cognition (neurodegenerative diseases other than FTD, major stroke, severe head injuries, mental retardation), alcohol and drug dependence, severe mental illness, and use of high-dose psychoactive medications were excluded from the analysis.[3] Non-Italian speaking incident patients were also excluded. None of the study participants showed oxygen saturation <92% based on pulse oximetry at the time of their neuropsychological assessment. Six hundred seventy-seven healthy controls, matched by age, sex, and ancestry (based on the origin of both parents), were recruited from the patients' general practitioners. Out of 1313 incident cases in the period 2007-2015, 751 ALS patients from the participant ALS Centers (57.2%) underwent whole-genome sequencing and full neuropsychological assessment.

Neuropsychological evaluation

An extensive list of neuropsychological tests was performed on each patient.[4] These included: Mini-Mental State Examination (MMSE); Letter and category fluency tests; Frontal Assessment

Battery (FAB); Digit Span Forward and Backward; Trail-Making Test (TMT) A and B; Rey Auditory Verbal Learning Test (RAVLT), immediate and delayed recall; Babcock Story Recall Test (BSRT), immediate and delayed recall; Rey-Osterrieth Complex Figure (ROCF), copy and delayed recall; and Raven's Colored Progressive Matrices (CPM47). Neurobehavioral dysfunction was determined by patient history, direct observation by the neuropsychologist, and the family form of the Frontal Systems Behavior Scale completed by a close relative or caregiver. The Edinburgh Cognitive and Behavioral ALS Screen (ECAS) was not included in the battery since the Italian validation was published in 2018.[5] Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS); the item "I feel slowed down" was discussed with patients in order to have them not to refer to physical disability.[3] The raw data of the neuropsychological tests were adjusted for age and years of education according to the Italian normative. The battery was administered following the same sequence to avoid differential interference. All study participants were tested at diagnosis or during the first follow-up visit (2 months later).

Cognitive categorization

Patients' cognitive status was classified according to the revised ALS-FTD Consensus Criteria[6] into five categories:

1. ALS with normal cognition (ALS-CN);
2. ALS with behavioral impairment (ALSbi);
3. ALS with cognitive impairment (ALSci);
4. ALS with cognitive and behavioral impairment (ALScbi)

5. ALS with FTD (ALS-FTD).

Patients were retrospectively classified blindly by two neuropsychologists with expertise in ALS (BI, LP). The concordance rate was over 90% for all diagnoses. When there was disagreement, the case was discussed until a consensus diagnosis was established.[4] In the analyses the ALSbi, ALSci and ALScbi group were collapsed into a single group, i.e. intermediate cognitive phenotype.

Whole-genome sequencing

All eligible patients and healthy subjects were screened for *GBA* variants through whole-genome sequencing (WGS). Blood samples and genomic DNA have been processed according to standard protocols: library preparation and read sequencing have been performed as per manufacturer protocol; read alignment, variant calling and quality control have been performed according to standard protocols. WGS methods have been fully described elsewhere[7]. Annotation was performed with KGGseq v1.0 (pgmlab.top/kggseq). A repeat-primed PCR determined the presence of the *C9orf72* hexanucleotide expansion. A threshold of ≥30 repeats with the typical sawtooth pattern was considered pathological.[8]

Statistical Analysis

Descriptive statistics were calculated for baseline demographics and cognitive testing. Survival was calculated from onset to death or censoring date (December 31st, 2019) using the Kaplan-Meier method. Patients with tracheostomy were coded as deceased on the date of the procedure.

Comparisons were performed using the log-rank test. In the first step of the analyses, only variants known to be a risk factor for cognitive decline in PD were considered. A single-variant association test was used to compare the frequency of *GBA* variants between ALS cases and healthy subjects.[9] A binomial test was used to assess the prevalence of *GBA* mutations across cognitive groups. Given the strong influence of *C9orf72* on cognitive status, we performed the binomial test with and without *C9orf72* expansion carriers to rule out its impact on the results. Linear mixed-effects models were used to test for associations between *GBA* genotype and cognitive functioning. The covariates included in this model were age at onset, sex, site of onset (classified as bulbar or spinal), the presence of bulbar signs at diagnosis, the rate of ALS Functional Rating Scale-Revised (ALS-FRS-R) decline at the time of diagnosis, and *C9orf72* status. ALS-FRS-R decline was calculated as follows: (48 - ALS-FRS-R at diagnosis) / months from disease onset to diagnosis. All statistical analyses were performed in R v.3.6.0 (http://www.r-project. org). R scripts are available on GitHub (http://github.com). To assess whether pathogenic rare variants in *GBA* contribute to cognitive decline risk in ALS, a gene-based rare variants association test was also performed. This analysis provided an independent approach from the assumption that only *GBA* variants associated with PD may influence cognitive status. Only rare variants were included, and minor allele frequency threshold was set at < 5%. The analysis was performed confronting ALS patients with cognitive decline (i.e. ALS-FTD and ALSCi/Cbi/Bi) vs ALS patients with normal cognitive function. The rare variant burden was assessed using the Sequence Kernel Association Test (SKAT) and the Sequence Kernel Association Test - Optimized (SKAT-O) as implemented in RVtests (http://zhanxw.github.io/rvtests/).

Supplementary Tables

Supplementary Table 1. Genes and Risk Factors associated with Parkinson Disease (PD), Dementia with Lewy Bodies (DLB), frontotemporal dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) that have been implicated in Lysosomal function.

Gene	Disease	Functions involving lysosomes
SNCA	Early-onset PD, DLB	Recruitment of proteins to lysosomal damage sites
PARK2	Early-onset PD	Mitophagy
PINK1	Early-onset PD	Mitophagy
LRRK2	Late-onset PD	Regulattion of lysosomal pH and homesostasis, and lysosome-Golgi trafficking
GBA	Late-onset PD, DLB	Accumulation of cholesterol in lysosomes
VPS35	Late-onset PD	Endosome–Golgi trafficking; autophagy
C9ORF72	ALS/FTD	Autophagy induction and stress granule autophagy
GRN	FTD	Regulation of lysosomal pH
TBK1	ALS/FTD	Clearance of damaged mitochondria, recruitment to lysosomes by α-synuclein

OPTN	ALS/FTD	Clearance of damaged mitochondria, recruitment to lysosomes by α-synuclein
CHMP2B	ALS/FTD	Regulation of lysosomal trafficking
CHCHD2	PD	Mitochondrial quality control
CHCHD10	ALS/FTD	Mitochondrial quality control
VCP	ALS/FTD	Autophagosome maturation and clearance of damaged lysosomes
SQSTM1	ALS/FTD	It targets protein aggregates for lysosomal degradation

Supplementary Table 2. Results from the gene-based rare variant association test

Burden test	**FTD**	**ALS-Bi / ALS-Ci / ALS-Cbi**
SKAT	0.196	0.0125
SKAT-O	0.184	0.00005

Supplementary Table 3. List of *GBA* variants detected in our ALS series. We identified three common *GBA* polymorphisms (p.E365K, p.T408M, p.N409S), which are known risk factors for DLB and cognitive impairment in PD[10–13]. Six of the other variants have not been reported to be associated with PD.

Nucleotide	AA	Exon	SNP id	gnomAD NFE	CADD score	ClinVar	Disease
c.C1319T	p.P440L	10	rs74598136	.	26.2	Pathogenic	Gaucher's disease
c.G1279A	p.E427K	10	rs149171124	0.0003	23.2	.	.
c.A1226G	p.N409S	10	rs76763715	0.002	22.7	Pathogenic	Gaucher's disease, PD, DLB
c.C1223T	p.T408M	9	rs75548401	0.0091	22.2	Likely benign	PD
c.G1093A	p.E365K	9	rs2230288	0.0121	17.33	Pathogenic	PD
c.A928G	p.S310G	8	rs1057942	0	14.81	.	.
c.C740G	p.T247S	7	.	.	22	.	.
c.T634A	p.S212T	7	rs398123533	.	1.937	VUS	
c.G626A	p.R209H	7	rs749416070	3.92E-05	17.53	.[§]	Reported in PD[14]
c.A38G	p.K13R	3	rs150466109	0.0002	0.003	Benign	

AA: amino acid; SNP: Single Nucleotide Polymorphism; gnomAD NFE: exome frequency in Non-Finnish European.

§ Classified as likely pathogenic according to the following American College of Medical Genetic (ACMG) criteria[15]: PM1 moderate (Hot-spot of length 17 amino-acids has 7 non-VUS missense/in-frame variants, 6 pathogenic and 1 benign), PM2 moderate (GnomAD exomes homozygous allele count = 0 is less than 3 for recessive gene GBA), PM5 moderate (Alternative variants Arg209Pro and Arg209Cys are classified as Pathogenic), PP2 supporting (269 out of 282 non-VUS missense variants in *GBA* gene are pathogenic).

Supplementary Table 4. Comparison of *GBA* risk variants frequency between ALS patients and healthy controls. These variants were found in 18 ALS patients (2.40% of our cohort) and 15 healthy controls (2.22%). The variant frequencies observed in our Italian population are consistent with those reported in gnomAD. The single-variant analysis confirmed that *GBA* variants are not a risk factor for developing ALS. We did not observe homozygote cases or compound heterozygous cases for *GBA* variants. Furthermore, we did not observe mutations in ALS-related genes (*SOD1*, *TARDBP* and *FUS*) in *GBA* risk variants carriers. We identified one patient who carried both a *GBA* risk variant (p.N409S) and the *C9orf72* repeat expansion.

GBA variant	n. Heterozygous ALS cases (n=751)	n. Heterozygous HC (n=677)	p-value	gnomAD NFE
c.1093G>A (p.E365K)	3	5	0.49	0.0121
c.1223C>T (p.T408M)	8	6	0.61	0.0091
c.1226A>G (p.N409S)	7	4	0.76	0.002

Supplementary Table 5. Clinical and demographic characteristics of ALS patients grouped by *GBA* genotype. The *GBA* risk variants did not influence survival or the motor phenotype. Only two of the ALS patients carrying a *GBA* risk variant exhibited extrapyramidal signs. Both patients had slowly progressive disease, and they developed extrapyramidal signs years after the initial ALS diagnosis. None of the *GBA* risk variants carriers had a family history of Parkinson's disease or other neurodegenerative diseases. The effect of *GBA* mutations on cognitive but not motor phenotype could be explained by a lower susceptibility of motor neurons: it should be noted that while extrapyramidal involvement is established in *GBA* homozygous carriers, motor neuron involvement has only been recently reported in few cases with Gaucher's Disease and one subject with a heterozygous *GBA* mutation. [16,17]

	***GBA* carriers (n=18)**	***GBA* non carriers (n=733)**	**P**
N (% male)	5 (27.8%)	404 (55.1%)	0.04
Age onset (yrs, SD)	65.2 (10.5)	65.7 (9.9)	0.84
Bulbar onset, n (%)	4 (22.2%)	244 (33.3%)	0.46
Bulbar signs at diagnosis, n (%)	9 (50.0%)	369 (49.2%)	1
C9orf72 expansion carriers (%)	1 (5.6%)	66 (9.0%)	0.93
Median survival (years, SD)	2.65 (1.68-5.26)	3.26 (1.68-5.89)	0.43*
Mean ALSFRS-R slope	1.31 (1.21)	0.99 (1.74)	0.33

* Survival was calculated with Kaplan-Meier curves and significance with Log-rank test

Supplementary Table 6. Cognitive phenotypes of *GBA* risk variant carriers. Due to the relatively small number of *GBA* variant carriers, we could not properly draw any conclusion on whether *GBA* variants were predominantly associated with cognitive or behavioural impairment or with a distinct pattern of cognitive deficits. However, it should be noted that the only variant causing Gaucher's Disease of this group (p.N409S) was associated with cognitive impairment in 7 out of the 8 carriers (87.5%): it could be postulated that more disruptive variants have a larger influence on cognitive function. Longitudinal neuropsychological studies and the application of neuroimaging techniques might allow further clarification on this issue. As recently reported in cross-sectional study using ^{18}F-2-fluoro-2-deoxy-D-glucose-PET,[18] the extent of metabolic brain changes in ALS patients reflects the degree of cognitive impairment, paralleling brain metabolic alterations observed in FTD over time.[19]

GBA variant	FTD	ALSBi	ALSCi	ALSCbi	CN
c.626G>A (p.R209H)	0	0	1	0	0
c.1093G>A (p.E365K)	0	2	0	0	1
c.1223C>T (p.T408M)	3	0	2	0	3
c.1226A>G (p.N409S)	2 §	1	2	1	1

§ One subject carrying the p.N409S variant and presenting FTD also carried the *C9orf72* repeat expansion

References to Supplementary materials

1. Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders*. 2000;1(5). doi:10.1080/146608200300079536

2. Chiò A, Mora G, Moglia C, et al. Secular Trends of Amyotrophic Lateral Sclerosis. *JAMA Neurology*. 2017;74(9). doi:10.1001/jamaneurol.2017.1387

3. Montuschi A, Iazzolino B, Calvo A, et al. Cognitive correlates in amyotrophic lateral sclerosis: a population-based study in Italy. *Journal of Neurology, Neurosurgery & Psychiatry*. 2015;86(2):168-173. doi:10.1136/jnnp-2013-307223

4. Iazzolino B, Pain D, Peotta L, et al. Validation of the revised classification of cognitive and behavioural impairment in ALS. *J Neurol Neurosurg Psychiatry*. 2019;90(7):734-739. doi:10.1136/jnnp-2018-319696

5. Poletti B, Solca F, Carelli L, et al. Cognitive-behavioral longitudinal assessment in ALS: the Italian Edinburgh Cognitive and Behavioral ALS screen (ECAS). *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*. 2018;19(5-6):387-395. doi:10.1080/21678421.2018.1473443

6. Strong MJ, Abrahams S, Goldstein LH, et al. Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*. 2017;18(3-4). doi:10.1080/21678421.2016.1267768

7. Grassano M, Calvo A, Moglia C, et al. Mutational analysis of known ALS genes in an Italian population-based cohort. *Neurology*. Published online November 2020. doi:10.1212/WNL.0000000000011209

8. Renton AE, Majounie E, Waite A, et al. A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD. *Neuron*. 2011;72(2). doi:10.1016/j.neuron.2011.09.010

9. Lee S, Abecasis GR, Boehnke M, Lin X. Rare-Variant Association Analysis: Study Designs and Statistical Tests. *The American Journal of Human Genetics*. 2014;95(1):5-23. doi:10.1016/j.ajhg.2014.06.009

10. Mata IF, Leverenz JB, Weintraub D, et al. *GBA* Variants are associated with a distinct pattern of cognitive deficits in Parkinson's disease: Cognitive Profile in *GBA* -Related PD. *Mov Disord*. 2016;31(1):95-102. doi:10.1002/mds.26359

11. Greuel A, Trezzi J, Glaab E, et al. *GBA* Variants in Parkinson's Disease: Clinical, Metabolomic, and Multimodal Neuroimaging Phenotypes. *Mov Disord*. 2020;35(12):2201-2210. doi:10.1002/mds.28225

12. Davis MY, Johnson CO, Leverenz JB, et al. Association of *GBA* Mutations and the E326K Polymorphism With Motor and Cognitive Progression in Parkinson Disease. *JAMA Neurol*. 2016;73(10):1217. doi:10.1001/jamaneurol.2016.2245

13. Cilia R, Tunesi S, Marotta G, et al. Survival and dementia in GBA -associated Parkinson's disease: The mutation matters. *Ann Neurol*. 2016;80(5):662-673. doi:10.1002/ana.24777

14. Heijer JM, Cullen VC, Quadri M, et al. A LARGE-SCALE Full *GBA1* Gene Screening in Parkinson's Disease in the Netherlands. *Mov Disord*. 2020;35(9):1667-1674. doi:10.1002/mds.28112

15. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-423. doi:10.1038/gim.2015.30

16. Pozzilli V, Giona F, Ceccanti M, et al. A case of motor neuron involvement in Gaucher disease. *Molecular Genetics and Metabolism Reports*. 2019;21:100540. doi:10.1016/j.ymgmr.2019.100540

17. Oliveira LM, Rastin T, Nimmo GAM, et al. Occurrence of Amyotrophic Lateral Sclerosis in Type 1 Gaucher Disease. *Neurol Genet*. 2021;7(4):e600. doi:10.1212/NXG.0000000000000600

18. Canosa A, Moglia C, Manera U, et al. Metabolic brain changes across different levels of cognitive impairment in ALS: a ^{18} F-FDG-PET study. *J Neurol Neurosurg Psychiatry*. Published online November 23, 2020:jnnp-2020-323876. doi:10.1136/jnnp-2020-323876

19. Diehl-Schmid J, Grimmer T, Drzezga A, et al. Decline of cerebral glucose metabolism in frontotemporal dementia: a longitudinal 18F-FDG-PET-study. *Neurobiology of Aging*. 2007;28(1):42-50. doi:10.1016/j.neurobiolaging.2005.11.002