Mutation screening and burden analysis of GLT8D1 in Chinese patients with ALS

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Abstract

Objective Glycosyltransfersase 8 domain containing 1 (GLT8D1) gene was identified to be an amyotrophic lateral sclerosis (ALS) causative gene via pedigree co-segregation and burden analysis. However, validations based on large cohort of ALS among different ethnic population are essential. We aimed to systematically screen all exons of GLT8D1 in a large cohort of Chinese ALS patients, study the genotype-phenotype correlation and explore the role of rare variants of GLT8D1 in ALS.

Methods A total of 977 sporadic ALS (sALS) and 47 familial ALS (fALS) cases underwent whole exome sequencing. Rare variants with MAF<0.1% in GLT8D1 were analyzed. Moreover, by using the controls from gnomAD database, rare variants were included in the burden analysis via 5 different algorithms.

Results We identified 1 likely pathogenic variant in the exon 4 of GLT8D1 in a fALS case and validated within the pedigree. Moreover, 3 variant of uncertain significance (VUS) in 4 patients among the 977 sALS cases 1 VUS in 1 case among the 47 fALS cases were also identified. Furthermore, in the burden analysis, there were no significant enrichment of rare variants of GLT8D1 in the whole gene level or exon 4 exclusively among Chinese patients with sALS.

Conclusion Cosegregation findings in our study further supported the pathogenic role of GLT8D1 in fALS. However, no pathogenic mutation was identified in the sALS patients, and rare variants were not enriched in the whole gene level or exon 4 of GLT8D1 among sALS patients, both of which suggested that the GLT8D1 may not play a role in Chinese patients with sALS.

Background

Amyotrophic lateral sclerosis (ALS) is a progressive adult-onset neurodegenerative disease characterized by both upper and lower motor neuron loss in the brain and spinal cord, of which the death usually occurs within 3–5 years from onset, often resulting from respiratory failure[1]. The exact etiology and mechanism of ALS remain largely unknown. Previous studies have shown that genetic factors play important roles in the pathogenesis of ALS, since about 10% of ALS patients have a family history of ALS[2]. So far, more than 30 genes have been identified as ALS causative genes[3], which can account for approximately two-thirds of familial ALS (fALS) and about 10% of sporadic ALS (sALS)[4, 5]. However, the genetic etiology could not be identified even in some familial cases, which highlights the importance of the discovery of additional ALS causative genes.

In recent years, with the development of gene sequencing methods and bioinformatic tools, an increasing number of novel ALS causative genes have been identified. A missense mutation p.R92W located in the exon 4 of glycosyltransfersase 8 domain containing 1 gene (GLT8D1) was found to co-segregate with ALS in an autosomal dominant ALS family[6]. Furthermore, 5 additional rare deleterious mutations in the same exon were also identified in 14 ALS cases, which implied that the rare deleterious variants affecting conserved amino acids in exon 4 of GLT8D1 were significantly enriched in ALS patients[6]. Functional studies found overexpression of mutant GLT8D1 (p.R92W and p.G78W) decreased the enzyme activity which lead to in vitro cytotoxicity and induced motor deficits in zebrafish consistent with ALS[6]. Thus, GLT8D1 gene defined as a novel ALS causative gene in 2019[6]. However, in a subsequent replication study conducted in a cohort of 512 ALS patients from Southeast of China failed to identify any rare variant in the exon 4 of GLT8D1[7]. There are genetic heterogeneity between different ethnics and even subpopulation[8]. Moreover, there is no study systematically investigating the role of other exons of GLT8D1 in ALS. Therefore, in the current study, we aimed to: 1) systematically screen the mutations of all exons of GLT8D1 in a large cohort of Chinese ALS patients; 2) study the genotype-phenotype correlation; 3) explore the role of rare putative pathogenic variants of GLT8D1 in ALS via burden analysis.

Methods

Participants and clinical assessments

A total of 977 Chinese sALS patients and 47 fALS probands admitted to Department of Neurology, West China Hospital of Sichuan University were enrolled in the study. All the ALS patients were diagnosed based on the El Escorial revised criteria for definite or probable ALS[9]. fALS is defined as patients with an identifiable family history of ALS among the first, second or third degree relatives[10]. Disease severity was assessed with the Revised ALS Functional Rating Scale (ALSFRS-R) and disease progression was calculated as (48-ALSFRSR)/ disease duration (months). Written and signed informed consent were obtained from all the participants. The study was approved by the Ethics Committee of Sichuan University.

Whole exome sequencing

Genomic DNA was collected from peripheral blood leukocytes and underwent whole exome sequencing (WES). Regarding WES, A total of 5ug DNA was fragmented to an average size of 350 bp with a Covaris LE220-plus Focused-ultrasonicator, and DNA library was constructed with KAPA Library Amplification Kit according to the manufacture's instruction. Then WES was performed with NovaSeq 5000/6000 S2/S1 Reagent Kit (Illumina). Clean data was filtered with Trimmomatic-0.36 biotool with a criteria of Clean Ratio ≥ 98%; Q20 ≥ 90%; Q30 ≥ 85%. Clean data were mapped to the reference genome (hg19) to obtain the bam file with the Burrow-Wheeler Aligner software (with target area coverage > 90%; mapping rate > 99% and repetition rate < 15%). The Genome Analysis Toolkit (GATK) was applied to perform quality calibration on the bam file, such as Realign and Base-Recalibrator. Haplotype-Caller in GATK was used to analyze the quality- controlled bam file and obtain the vcf file.

GLT8D1 analysis

All patients included in the GLT8D1 analysis were excluded for other known ALS/FTD-causative genes, including SOD1, TARDBP, FUS, PFN1, SQSTM1 and CHCHD10 and so on[11]. C9orf72 were sequenced using Repeat-primed PCR and gel electrophoresis methods as previously described[12]. The average depth for GLT8D1 was over 100x. Considering the autosomal dominant-inherited model for GLT8D1, we selected the variants according to the minor allele frequency (MAF) have to be less than 0.1% in public database including 1000 Genome Project, the Exome Aggregation Consortium (ExAC), and the Genome Aggregation Database (gnomAD), as well as 1000 Chinese ancestry healthy controls (data from KINGMED company); and the pathogenicity was based on the in sillico prediction tools including SIFT and polyphen-2. Furthermore, for the candidate rare variants, sanger sequencing was performed.

Burden analysis for rare variants

The rare variant was defined by MAF lower than 0.1% in the gnomAD database. Controls were from gnomAD East Asian population. Five different algorithms method were used for burden analysis independently, including C-alpha Score Test (CALPHA)[13], Sequence Kernel Association Test (SKAT)[14], Sum of Squared Score U Statistic (SSU)[15], Rare-Variant Weighted Aggregate Statistic (RWAS)[8] and Combined Multivariate and Collapsing Method (CMC)[16].

Results

The demographic characteristics of all participants are listed in **Supplementary Table 1**. In total, 3 rare missense variants (p.H24R in the exon 3, p.R255Q in the exon 8 and p.G281S in the exon 9) in GLT8D1 were identified in 4 (4.1%) patients among the 977 sALS patients, and 2 (4.3%) rare missense variant (p.G78A in the exon 4 and p.V291I in exon 9) were identified in 2 index patients among the 47 fALS cases **(**Table 1 **and Fig. 1)**.

The pedigree validation of the fALS patient carrying p.V291I was not available because the patient's affected family member has died. The variant p.G78A co-segregated with ALS between a proband and his mother (PP1); moreover, only mutations in the exon 4 were considered as pathogenic[6] (PM1); furthermore, p.G78A was absent in the East Asian controls (PM2) but altered the amino acid residue at the same position as the variant-p.G78W, which has been reported to be pathogenic by the previous study[6] (PM5); last but not least, it was predicted to be damaging by in sillico tools (PP3). Therefore, this variant can be classified as likely pathogenic according to the guideline (PM1 + PM2 + PM5 + PP1 + PP3)[17]. While the remaining 4 variants were classified as variant of uncertain significance (VUS)[17].

The clinical characteristics of patients carrying rare variants in GLT8D1 was listed in Table 1. The average AAO of these 6 patients carrying variants in GLT8D1 was 55.4 years old (ranging from 44.1–66.6 years old). Most of these patients (4/6) had limb onset. The male patient carrying the likely pathogenic mutation p.G78A developed weakness in the right upper limb at the age of 44; at the time of enrollment, the patient had possible ALS with a disease duration of 7 months and a ALSFRS-R score of 47. The electrophysiological examination indicated neurodegeneration involved in the cervical segment. After 3 months, the electrophysiological examination revealed cervical, thoracic and lumbosacral segments involvement. However, weakness remained in the right upper limb. The patient had normal cognition, frontal function as well as psychiatric status. The proband's mother was found to carry the same mutation and had weakness of the left upper arm and interosseus muscle atrophy for 5 years but the symptoms progressed very slowly. Furthermore, the electrophysiological examination indicated neurodegeneration involved in the cervical segment.

To further evaluate the accumulated association of the rare variants in GLT8D1 with ALS, we did whole-gene level and exon 4 specific rare variants burden analyses in sporadic cases. In the whole gene level, together with previous study from China mainland[7], there were totally 4 rare variants (5 alleles) among the 1410 sALS (2820 alleles); while there were 25 variants (54 alleles) fulfilled the same criteria among 9766 cases (19532 alleles) in the gnomAD East Asian controls. Furthermore, in the exon 4, there was no rare putative pathogenic variant (0 allele) among the 1410 sALS (2820 alleles); while there were 3 rare variants (5 alleles) fulfilled the same criteria among 9766 cases (19532 alleles) in the gnomAD East Asian controls. Among the 5 algorithms analyzing disease burden, there were no significant enrichment of putative pathogenic variants in our study when compared with the gnomAD East Asian controls either in the whole gene level or exon 4 level exclusively **(Supplementary Table 2)**.

Discussion

As far as we know, the current study was the largest study systematically investigating the role of GLT8D1 in ALS in a Chinese population. We identified 1 likely pathogenic variant in the exon 4 of GLT8D1 in a fALS case and validated within the pedigree. Moreover, in the current study, 3 VUS in 4 patients among the 977 sALS cases 1 VUS in 1 case among the 47 fALS cases were also identified. Furthermore, in the burden analysis, there were no significant enrichment of rare variants in the whole gene level or exon 4 exclusively among Chinese patients with sALS.

GLT8D1 was recently identified to be causative gene for ALS via pedigree co-segregation[6]. All mutations GLT8D1 were identified in exon 4 of GLT8D1 via a haploinsufficiency mechanism of toxicity. GLT8D1 belongs to the glycosyltransferase family 8 and involve in the transfer of a glycosyl group from a donor to an acceptor via a "retaining" mechanism[18]. And disrupted glycosylation of lipids and proteins has been strongly linked with neurodegeneration via 2 prominent glycosyltransferase-associated mechanisms: ganglioside synthesis and addition of O-linked b-N-acetyl-glucosamine to proteins (O-GlcNAcylation)[18]. Ganglioside synthesis is functioning prominently in cell signaling and O-GlcNAcylation is crucial for axonal and synaptic function, both of which have been found to be dysregulated in animal models and patients of neurodegenerative diseases including Parkinson's disease, Huntington's disease, Alzheimer's disease and ALS[18].

In the current study, only 1 likely pathogenic mutation-p.G78A in the exon 4 of GLT8D1 was identified in 1 index case of familial ALS. Previous study suggested that a rare deleterious p.G78W variant in the exon 4 of GLT8D1 did a common pathogenic effect as p.R92C mutation in the exon 4 of GLT8D1[6]. Our patient carrying p.G78A mutation had an early age of onset and spinal onset. And his symptoms were mild at the disease duration of 10 months. Furthermore, the mother of this patient had very mild symptoms and slow progression. The previous study reported that the patient carrying p.G78W had a relatively longer survival of more than 5 years[6]. These evidences indicated that amino acid change at site 78 might be indicatable for longer survival. However, it is important to follow-up the progression of this patient before we made a conclusion that p.G78A mutation cause mild phenotype.

Burden analysis is a method assessing the accumulated effect of a gene on a single disease. However, there were no significant differences when comparing the rare variants found in Chinese patients with sALS with gnomAD East Asian controls both in the whole gene level and exon 4 level of GLT8D1 exclusively, indicating that rare deleterious variants in GLT8D1 might not be explicitly relevant to Chinese patients with sALS. It was not consistent with the finding of the original study[6]. This further emphasize genetic heterogeneity between different ethnics. However, we have to acknowledge the limitation of our current study, where we used East Asian population in gnomAD as our control but not the matched control from the same region, while rare variants can be (sub)population specific. Therefore, more studies from other ethnics would be extremely needed to further clarify the role of GLT8D1 in the pathogenesis of sALS.

Conclusion

Co-segregation findings in our study further supported the pathogenic role of GLT8D1 in fALS. However, no pathogenic mutation was identified in the sALS patients, and rare putative pathogenic variants were not enriched in the whole gene level or exon 4 of GLT8D1 among sALS patients, both of which suggested that the GLT8D1 may not play a role in sALS in a Chinese population.

Abbreviations

Full name	Abbreviations
Glycosyltransfersase 8 domain containing 1	GLT8D1
Amyotrophic lateral sclerosis	ALS
sporadic ALS	sALS
familial ALS	fALS
uncertain significance	VUS
the Revised ALS Functional Rating Scale	ALSFRS-R
Whole exome sequencing	WES
Genome Analysis Toolkit	GATK
Minor allele frequency	MAF
the Exome Aggregation Consortium	ExAC
the Genome Aggregation Database	gnomAD
C-alpha Score Test	CALPHA
Sequence Kernel Association Test	SKAT
Sum of Squared Score U Statistic	SSU
Rare-Variant Weighted Aggregate Statistic	RWAS
Combined Multivariate and Collapsing Method	CMC
O-linked b-N-acetyl-glucosamine to proteins	O-GlcNAcylation

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all participants. This study was approved by the ethics committee of Sichuan University.

Consent for publication

All participants were properly consented for this study.

Availability of data and materials

The data supporting the conclusions of this article are included within the article and the supplementary files.

Competing interests

All the authors declared that they have no competing interests

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Authors' contributions

CB, XJG and HFS conceived and designed the study. BC wrote, XJG and HFS edited the manuscript. CYL and YPC performed the statistics analysis. RWO, BC, QQW, YBH, LYZ, WS, BZ, YW and XPC collected the clinical data. All authors read and approved the final manuscript.

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Tables

Table 1. Clinical characteristics of patients with pathogenic/like pathogenic variants in GTL8D1.

Description	dbSNP	ExAC East Asia	gnomAD East Asia	HGVS	SIFT	Polyphen2	ACMG classification	AAO (years)	Sex	Disease duration(month)	Family history	Site of onset	ALSFRS	Progression (score/month)	Survival time (months)
p.G78A	rs760081278	0	0	[DM]	D	D	Likely pathogenic	44.1	M	10.0	+	UL	47	0.14	10.0 (alive)
p.V291I	-	-	-		D	D	VUS	47.0	M	4.4	+	LL	43	1.14	10.0(died)
p.H24R	-	-	-		T	B	VUS	56.0	M	7.7	-	UL	45	0.39	7.1(died)
p.H24R	-	-	-		T	B	VUS	66.0	F	21.3	-	Bulbar	37	0.52	78.1(died)
p.R255Q	-	-	-		T	B	VUS	66.6	F	12.2	-	Bulbar	37	0.90	23.3(alive)
p.G281S	rs751719596	0.0002	0.00017		T	P	VUS	52.4	F	27.1	-	UL	19	1.01	18.1(alive)

UL: Upper limb; LL: Lower limb.

Figures

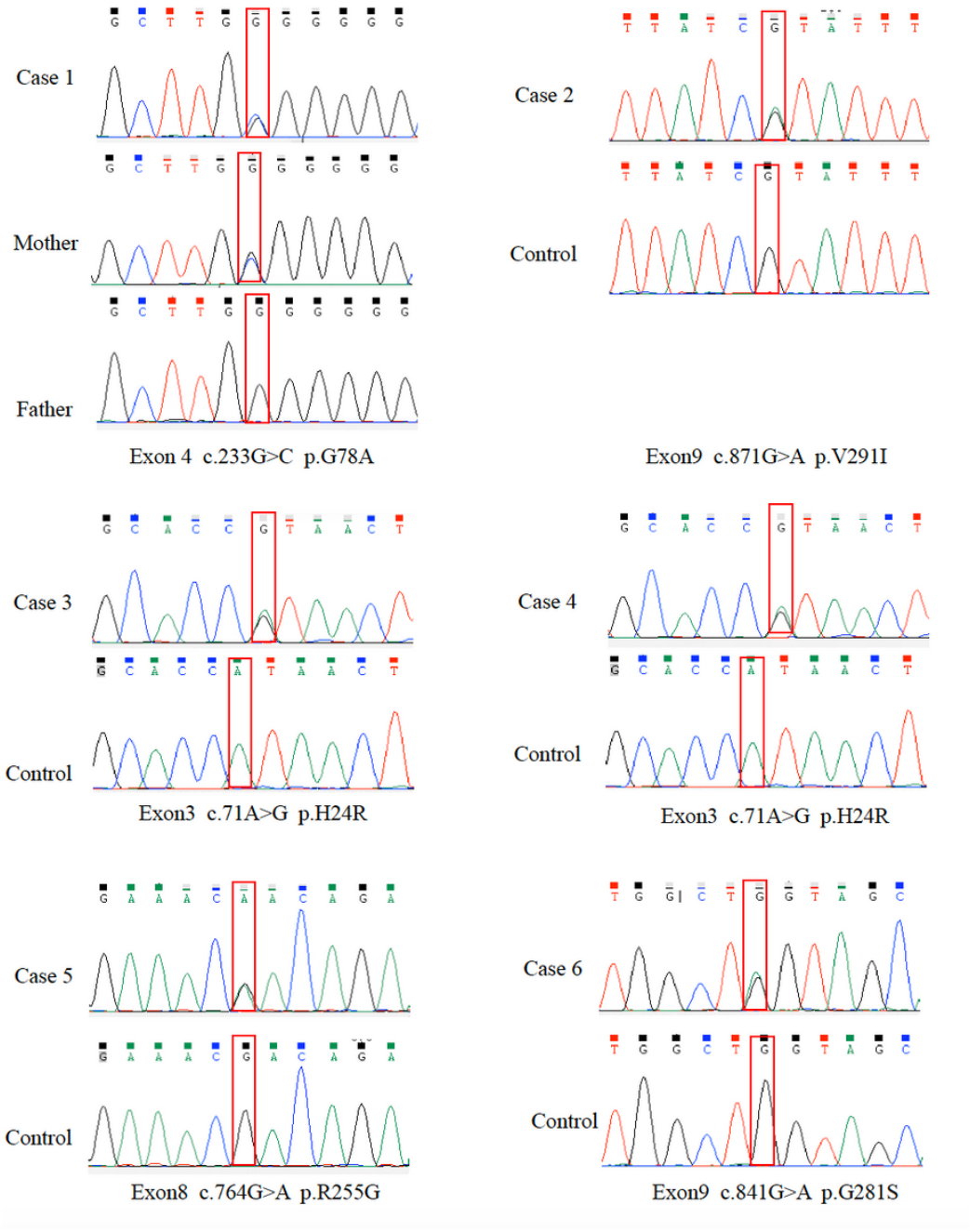

Figure 1

Sanger sequencing for the rare variants in GLT8D1.

Supplementary Files

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- Supplementarytable1.docx