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Genetic and Functional Analysis of Glycosyltransferase 8 Domain–Containing Protein 1 in Taiwanese Patients With Amyotrophic Lateral Sclerosis

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Abstract

Background and Objectives

To investigate the frequency, spectrum, and molecular functional effect of glycosyltransferase 8 domain-containing protein 1 (GLT8D1) variations in Taiwanese patients with amyotrophic lateral sclerosis (ALS).

Methods

We performed genetic analyses of *GLT8D1* in 410 unrelated patients with ALS by Sanger sequencing. The 410 patients were selected from a cohort of 477 unrelated patients with ALS after excluding variations in common ALS disease genes. Functional effects of the *GLT8D1* variation were investigated by in vitro functional analysis.

Results

We identified a novel heterozygous missense variation in *GLT8D1*, p.I290M (c.870C>G), in 1 single patient with familial ALS. The patient with the p.I290M variation had a spinal-onset ALS with disease onset at age 60 years and a survival of 6 years. Functional studies demonstrated that the variant I290M GLT8D1 protein was mislocalized to the endoplasmic reticulum (ER), provoked ER stress and unfolded protein response, compromised the glycosyltransferase activity, and led to an increased cytotoxicity.

Discussion

GLT8D1 variations account for 0.2% (1/477) of the patients with ALS in Taiwan. These findings expand the spectrum of *GLT8D1* variation and support the pathogenic role of *GLT8D1* variations in ALS.

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Glossary

ALS = amyotrophic lateral sclerosis; **C9ORF72** = chromosome 9 open reading frame 72; **CCK-8** = cell counting kit-8; **ER** = endoplasmic reticulum; **FUS** = fused in sarcoma; **GLT8D1** = glycosyltransferase 8 domain-containing protein 1; **gnomAD** = genome aggregation database; **LDH** = lactate dehydrogenase; **MPZ** = myelin protein zero; **RT-qPCR** = real-time quantitative PCR; **SOD1** = superoxide dismutase 1; **sXBP1** = spliced X-box–binding protein 1; **TARDBP** = TAR DNA-binding protein; **TBK1** = TANK-binding kinase 1; **UPR** = unfolded protein response; **WT** = wild-type.

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by relentless loss of upper and lower motor neurons, leading to progressive muscle weakness and atrophy with a short survival of 3–5 years.[1] Although the underlying causes of most patients with ALS are still unclear, approximately 10% of patients with ALS inherit their disease from their parents, suggesting that genetic factors have an important role in ALS pathogenesis. To date, more than 50 genes have been implicated in ALS pathogenesis, and variations in at least 10 of these genes have been clearly demonstrated to cause familial ALS.[2,3] Moreover, only superoxide dismutase 1 (SOD1), fused in sarcoma (FUS), transactive response DNA binding protein (TARDBP), chromosome 9 open reading frame 72 (C9ORF72), valosin-containing protein (VCP), and TRAF family member-associated NF-κB activator binding kinase 1 (TBK1) variations account for a significant number of patients with ALS, indicating a high degree of genetic heterogeneity of ALS.[2,4,5] However, the roles of newly identified causal genes of ALS, such as *GLT8D1*,[6] are yet to be completely understood because the relevant studies are still sparse.

The *GLT8D1* gene encodes glycosyltransferase 8 domain-containing protein 1 (GLT8D1), a single-pass transmembrane protein of 371 amino acids in length. GLT8D1 is a member of the glycosyltransferase family 8 and functions in transferring a glycosyl group from a donor to an acceptor molecule.[7] Recently, 1 British study identified *GLT8D1* variations in patients with familial or sporadic ALS.[6] They first identified a *GLT8D1* missense variation in exon 4, p.R92C, cosegregating with the disease in an autosomal dominant manner in the ALS family.[6] Then, 4 additional rare deleterious variations in *GLT8D1* exon 4 were further found in 4 patients with ALS. Statistical analysis showed that the rare deleterious variants affecting the conserved amino acids in exon 4 of *GLT8D1* were significantly enriched in patients with ALS.[6] Further functional studies revealed that R92C and G78W GLT8D1 variant proteins exhibited impaired glycosyltransferase activity, produced cytotoxicity, and led to motor deficits in zebrafish.[6] Another Chinese study screened *GLT8D1* variations in 977 patients with sporadic ALS and 47 patients with familial ALS and identified 1 likely pathogenic variant, p.G78A, in 2 patients within the same family.[8] Another 2 Chinese studies and 1 Australian study screened 512, 539 and 699 patients with ALS for *GLT8D1* variations, respectively, but failed to identify any pathogenic variation.[9-11]

To further understand the role of *GLT8D1* variations in ALS, we screened 410 unrelated Taiwanese patients with ALS for *GLT8D1* variations. In addition, in vitro studies were conducted to assess the functional effects of the variant gene product.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Informed consent was obtained from all patients in this study. The protocols for this study were approved by the Institutional Review Board of Taipei Veterans General Hospital.

Patients

Four hundred ten unrelated individuals (248 men and 162 women) with the diagnosis of probable or definite ALS based on the revised EL Escorial criteria were enrolled into this study.[12] All participants were of Han Chinese descent and were recruited from the Neurology Service of Taipei Veterans General Hospital, which is a 2,974-bed tertiary medical center that serves both veterans and regular citizens in Taiwan. It accepts both self-referred patients and referrals of difficult cases from other hospitals. These 410 patients were selected from a consecutive series of 477 unrelated patients with ALS after excluding variations in SOD1 (20 patients), C9ORF72 (18), TARDBP (16), FUS (8), cyclin-F (2), Optineurin (1), Matrin 3 (1), and TBK1 (1). Among the 410 patients with ALS, the average age at onset was 55.8 years (range 15–89). Eleven patients (2.7%) had a positive family history of ALS (familial ALS), and 399 (97.3%) were apparently sporadic cases. Seventy-seven patients (18.8%) suffered from bulbar-onset ALS, and 211 (51.5%) had an upper limb-onset disease.

Genetic Analyses

Genomic DNA was extracted from peripheral blood cells. Genetic analysis of the coding exons and their flanking regions of *GLT8D1* was performed by PCR amplification and Sanger sequencing with the intronic primers using the BigDye 3.1 dideoxy terminator methods with an ABI Prism 3700 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA). Amplicon sequences were compared with the reference *GLT8D1* coding sequence (NM_152,932.2). The sequence variations were validated by sequencing both sense and antisense strands of the amplicons. The putative pathogenic *GLT8D1* variants were first discriminated by absence in the 2 population databases, the genome aggregation database (gnomAD)[13] and Taiwan Biobank database (taiwanview.twbiobank.org.tw). In silico prediction of the pathogenicity of

the variant was conducted using 2 computational programs, MutationTaster[14] and PolyPhen-2.[15] Evolutionary conservation of the mutated amino acid residue was assessed by aligning the amino acid sequences of GLT8D1 orthologs of different species using the UniProt website (www.uniprot.org).[16]

Expression Plasmids, Cell Cultures, and Transfection

A full-length coding region of *GLT8D1* was cloned into pFLAG-CMV-5a (Sigma-Aldrich, St. Louis, MO) to generate the wild-type (WT) GLT8D1 expression construct. The 2 *GLT8D1* variations, p.I290M (c.870C>G) and p.R92C (c.274 G>A), were separately introduced into the WT expression plasmids by using the QuikChange Site-Directed Mutagenesis method (Agilent, Santa Clara, CA). The endoplasmic reticulum (ER) marker pDsRed-ER and the Golgi marker pDsRed-Monomer-Golgi were purchased from Clontech (Mountain View, CA). HEK293T cells were maintained in Dulbecco modified eagle medium supplemented with 10% FBS. Transient transfections were performed using Lipofectamine 2000 (Thermo Fisher Scientific).

Western Blot Analysis of the Steady-State Protein Levels

GLT8D1-transfected HEK293T cells were lysed in RIPA buffer, fractionated on 10% SDS-PAGE, and analyzed by Western blotting with anti-FLAG (#8146; Cell Signaling, Danvers, MA) or anti-actin (Merck Millipore, Burlington, MA) antibodies. Detection was performed with a standard enhanced chemiluminescence method.

Immunofluorescence Analyses

GLT8D1-transfected HEK293T cells were fixed with 4% paraformaldehyde followed by permeabilization with 0.2% tween-20. After blocking with 1% BSA, the cells were stained for GLT8D1 using the anti-FLAG antibody conjugated to Alexa 488 together with DAPI for counterstaining cell nuclei. The confocal images were acquired under a FluoView FV10i confocal microscope (Olympus, Tokyo, Japan).

Assessing ER Stress by Real-Time Quantitative PCR

To investigate the effect of the *GLT8D1* variations on ER stress, the mRNA expression levels of the ER stress biomarkers, binding immunoglobulin protein (BiP), C/EBP homologous protein (CHOP), and spliced X-box–binding protein 1 (sXBP1), were examined in the *GLT8D1*-transfected HEK293T cells by using real-time quantitative PCR (RT-qPCR). Total RNA of transfected cells was extracted using the RNeasy mini kit (Qiagen, Hilden, Germany). The cDNA synthesis was performed with the SuperScript1III 1st strand synthesis kit (Thermo Fisher Scientific). The RT-qPCR reactions were performed with SYBR Green master mix using a 7500 Fast Real-Time PCR System (Thermo Fisher Scientific). The relative gene expression was normalized against glyceraldehyde 3-phosphate dehydrogenase expression.

Glycosyltransferase Activity Assays

GLT8D1-transfected HEK293T cells were lysed in IP lysis buffer (Thermo Fisher Scientific). The lysates were incubated with anti-FLAG antibody-conjugated Dynabeads (Thermo Fisher Scientific) at 4°C overnight. Then, beads were washed with lysis buffer, and the pulled-down GLT8D1 proteins were eluted with 0.2 M glycine (pH 2.6) then neutralized with 1 M Tris-HCl (pH 9.0). Glycosyltransferase activity was measured using a glycosyltransferase activity kit (R&D Systems, Minneapolis, MN). Briefly, reactions were initiated by adding 10 ng/μL of purified GLT8D1 proteins to a reaction mixture including 5 mM UDP-galactose, 5 mM GlcNAc, and 2 ng/μL coupling phosphatase. Reactions were incubated at 37°C for 1 hour and then terminated by addition of Malachite Green reagent and OD620 read.

Cell Viability and Cytotoxicity Assays

Cell counting kit-8 (CCK-8) assay (Enzo Life Sciences, Farmingdale, NY) was used to assess the cell viability. *GLT8D1*-transfected cells were grown in 96-well plates, and CCK-8 solution was added to growing cultures and incubated at 37°C for 2 hours. The absorbance was measured at 450 nm using a microplate reader (Molecular Devices, San Jose, CA). Lactate dehydrogenase (LDH) activity was measured in the culture medium as an index of cytotoxicity, using the CyQUANT LDH cytotoxicity assay (Thermo Fisher Scientific). Culture medium of the transfected cells was transferred to a 96-well plate and incubated with the reaction mixture from the kit for 30 minutes. Absorbance at 490 and 680 nm was measured to determine LDH activity.

Data Availability

The data that support the findings of this study are available from the corresponding author. Data will be shared on reasonable request and after ethics approval if requested by other investigators.

Results

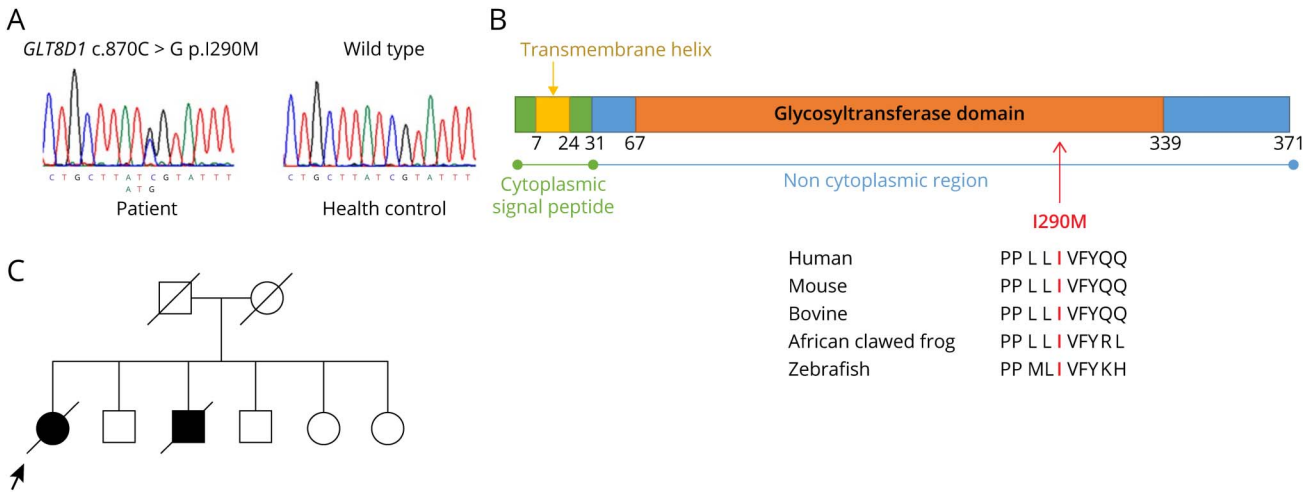
Identification of the Novel *GLT8D1* Variation

Genetic analyses of *GLT8D1* in the 410 patients with ALS revealed 1 heterozygous missense variant, p.I290M (c.870C>G) (Figure 1A), in 1 single patient with familial ALS. *GLT8D1* p.I290M was not found in the gnomAD as well as Taiwan Biobank database, which contains 1,517 Taiwanese healthy control exomes. The p.I290M variation alters an amino acid residue of GLT8D1 which is evolutionarily conserved from human to zebrafish (Figure 1B). PolyPhen2 and MutationTaster predicted the GLT8D1 p.I290M variant to be probably damaging (scores 1 for HumDiv and 0.999 for HumVar) and disease causing (probability 0.998), respectively.

I290M GLT8D1 Variant Protein Is Aberrantly Retained in the ER

GLT8D1 p.R92C was a well-confirmed pathogenic variation for ALS[6] and was used as a positive control in this study. To

Figure 1 *GLT8D1* p.I290M Variation Identified in This Study



(A) Sanger sequencing traces demonstrating the heterozygous *GLT8D1* c.870C>G (p.I290M) variation. (B) The domain diagram of human GLT8D1, the location of the variation identified in this study, and the alignment of multiple GLT8D1 orthologs showing conservation of the I290 residue from human to zebrafish. (C) Pedigree of the patient carrying the *GLT8D1* c.870C>G (p.I290M) variation. GLT8D1 = glycosyltransferase 8 domain-containing protein 1.

assess the functional effect of the *GLT8D1* p.I290M variation, we first investigated whether this variation could affect GLT8D1 protein expression. Western blot analysis revealed that the steady-state expression of the I290M GLT8D1 was much increased than that of the WT and the R92C GLT8D1 (Figure 2A). We then performed immunofluorescence analyses to demonstrate the intracellular distribution of the GLT8D1 proteins. As shown in Figure 2B, cells expressing WT or R92C GLT8D1 displayed discrete punctate perinuclear localization. Combined staining of GLT8D1 with a Golgi marker revealed that WT or the R92C variant protein signals overlapped largely with the Golgi marker (Figure 2C), implicating that WT or R92C GLT8D1 was localized primarily in the Golgi apparatus. However, staining of I290M GLT8D1 displayed abnormal reticular pattern throughout the cytoplasm rather than in the Golgi apparatus (Figure 2B). Combined labeling of I290M GLT8D1 with the ER marker showed a significant overlap between an ER marker and the I290M protein signals (Figure 2D), suggesting that the I290M GLT8D1 was predominantly retained in the ER instead of being transported to the Golgi apparatus.

I290M GLT8D1 Induces ER Stress and Unfolded Protein Response

To examine whether the I290M GLT8D1 could induce ER stress and unfolded protein response (UPR), we measured the mRNA levels of several ER stress genes in HEK293T cells transfected with the WT or variant *GLT8D1* constructs. Cells expressing R98C myelin protein zero (MPZ), a variant protein known to induce ER stress,[17] were used as positive control. As shown in Figure 3A, cells expressing I290M GLT8D1, as well as the positive control R98C MPZ, had significantly increased levels of ER stress markers, such as BiP, CHOP, and sXBP1, compared with cells expressing WT GLT8D1, indicating that I290M GLT8D1 induced ER stress. Cells expressing R92C GLT8D1 did not trigger UPR as significantly as I290M did.

I290M GLT8D1 Compromises the Glycosyltransferase Activity

To assess the influence of the p.I290M variation on glycosyltransferase activity, we purified the FLAG-tagged GLT8D1 proteins from HEK293T cells expressing WT or variant GLT8D1 constructs using immunoprecipitation. The cell-free enzymatic activity assays demonstrated that the glycosyltransferase activities were significantly reduced in the I290M GLT8D1 and R92C GLT8D1 compared with the activity of the WT protein (Figure 3B). These findings indicated that the *GLT8D1* p.I290M variation compromised the glycosyltransferase activity, which may subsequently perturb cellular lipid and protein synthesis.

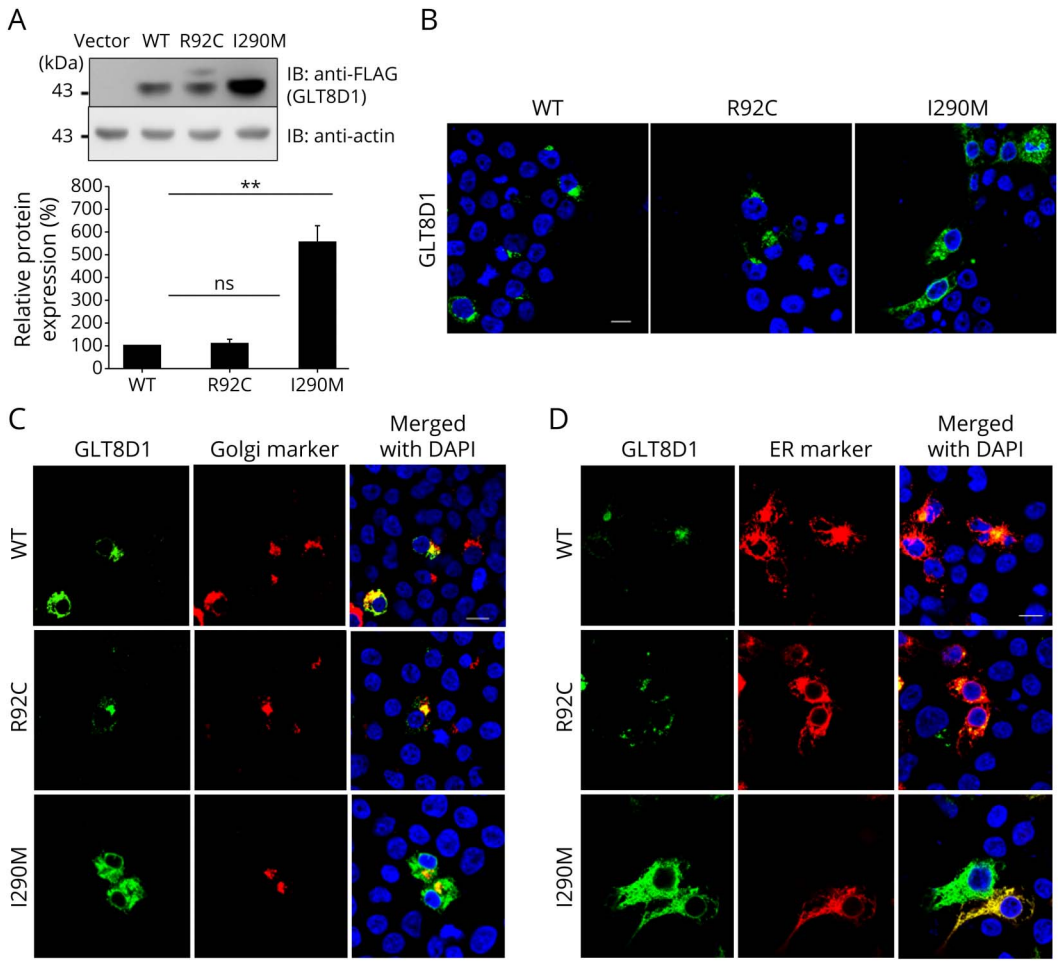
I290M GLT8D1 Leads to an Increased Cytotoxicity

Moreover, to determine whether the I290M variant protein may cause cell toxicity, CCK-8 assays were used to assess the cell viability of the *GLT8D1*-transfected cells while LDH assays were used to evaluate cell death. As shown in Figure 3C and D, compared with WT GLT8D1, both R92C and I290M variants had a significantly increased cellular toxicity in HEK293T cells.

Clinical Information of the Patient Carrying the *GLT8D1* Variation

The pathogenicity of *GLT8D1* p.I290M has been supported by both genetic analyses and in vitro functional studies. The patient harboring the *GLT8D1* p.I290M variation had an initial symptom of right hand atrophy and weakness at age 60 years. Then, the symptoms progressed to bilateral upper limbs and then lower limbs within 2 years. She developed dysarthria

Figure 2 In Vitro Expression of the GLT8D1 Variants in HEK293T Cells



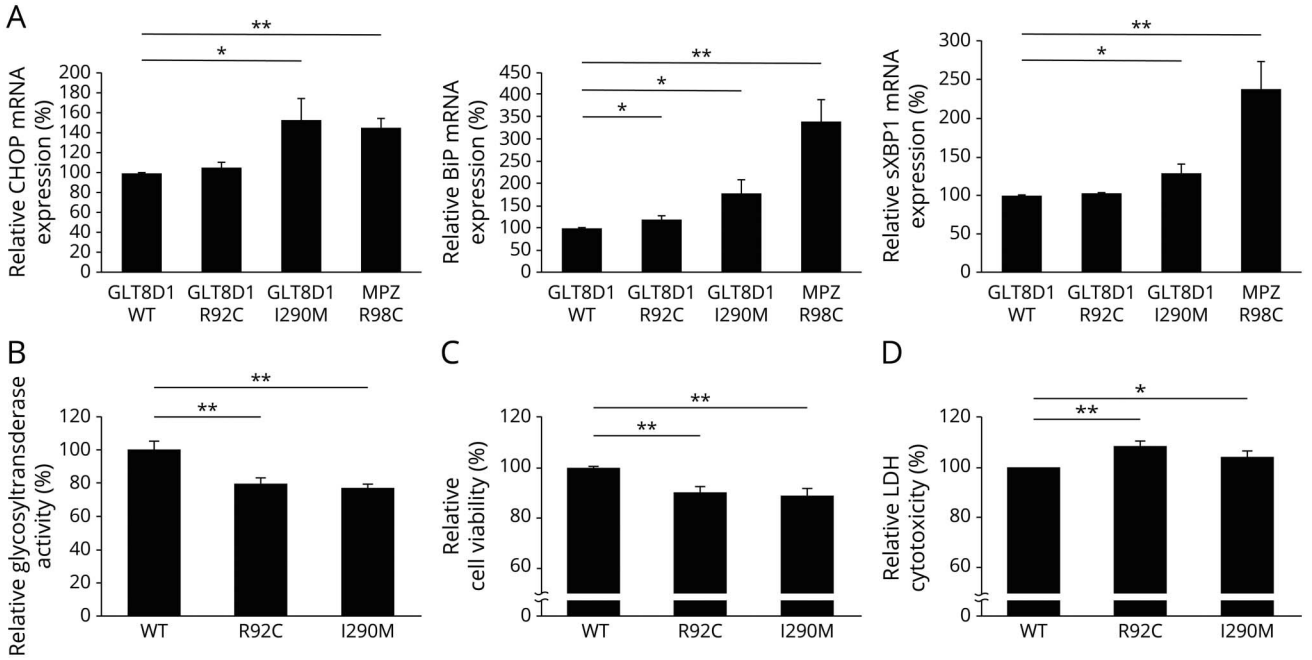
(A) Representative Western Blot analysis of steady-state expression of *GLT8D1* proteins in HEK293T cells transfected with *GLT8D1* constructs. Actin was used as a loading control. Densitometric quantification is shown below. The error bars indicate standard error of the mean (SEM) from 4 independent experiments. The asterisk indicates a statistically significant difference (**$p < 0.01$). (B) Immunofluorescence analyses of HEK293T cells expressing GLT8D1 mutants. Confocal fluorescence images of transfected cells labeled with an Alexa 488-conjugated anti-FLAG antibody (GLT8D1; green). Cell nuclei were stained with DAPI (blue). (C and D) Subcellular localization of the GLT8D1 mutants. Confocal fluorescence images of HEK293T cells cotransfected with construct encoding GLT8D1 (labeled with FLAG antibody, green) and markers of the Golgi (DsRed-Monomer-Golgi, red) or the ER (DsRed-ER, red). Cell nuclei were stained with DAPI (blue). Scale bar, 10 μm. ER = endoplasmic reticulum; GLT8D1 = glycosyltransferase 8 domain-containing protein 1; ns = means no statistically significant difference; SEM = standard error of the mean; WT = wild-type.

and dysphagia at age 64 years and received percutaneous endoscopic gastrostomy at age 65 years. Physical examination at age 65 years revealed tongue atrophy with fasciculation, severe dysarthria, and weakness and atrophy with fasciculation in 4 limbs (muscle strength of 1–2/5 according to the Medical Research Council scale), brisk deep tendon reflexes, spasticity over the lower limbs, and bilateral extensor plantar responses. She died of respiratory failure at age 66 years. Her younger brother was reported to die of ALS at age 30 years without additional clinical information (Figure 1C).

Discussion

GLT8D1 was recently identified as an ALS disease gene, and all the disease-associated variations were found in the exon 4.[6] Functional studies showed that the p.R92C and p.G78W variations could result in impaired GLT8D1 glycosyltransferase function and contribute to in vitro cytotoxicity and motor deficits in zebrafish.[6] To understand the contribution of *GLT8D1* variations to ALS in our population, we investigated *GLT8D1* in 410 unrelated Taiwanese patients with ALS and identified 1 novel heterozygous missense variation, p.I290M in 1 patient with familial ALS. This variation locates in exon 9 of *GLT8D1* and is predicted to alter a highly conserved amino acid residue within the glycosyltransferase domain of GLT8D1 (Figure 1B). Several lines of evidence support the pathogenicity of the *GLT8D1* p.I290M variation. First, it was present in a patient with familial ALS and was absent in gnomAD and Taiwan biobank databases. Second, it has been predicted as a damaging or disease causing variant by PolyPhen2 and MutationTaster programs. Furthermore, in vitro functional studies revealed that the p.I290M variation caused mislocalization of the variant GLT8D1 proteins to ER, provoked ER stress and UPR, compromised the glycosyltransferase activity, and led to an increased cytotoxicity. According to the American College

Figure 3 In Vitro Functional Analysis of the GLT8D1 Variants



(A) The activation of ER stress was evaluated by RT-qPCR, measuring the expression levels of CHOP, BiP, and sXBP1 mRNA. Cells transfected with R98C MPZ expression plasmid represented the positive controls. All values (mean ± SEM, n = 4) were normalized to the *GAPDH* mRNA levels. (B) Analyses of glycosyltransferase activities of the purified GLT8D1 proteins. FLAG-tagged WT and mutant GLT8D1 proteins were overexpressed in HEK293T cells and purified by immunoprecipitation. The inorganic phosphate generated through glycosyltransferase reactions were quantified to estimate the glycosyltransferase activities (n = 8). (C and D) Expression of I290M GLT8D1 and R92C GLT8D1 impaired cell viability (C) and induced cytotoxicity (D) in HEK293T cells compared with the WT proteins. Values are shown as means ± SEM of 8 independent transfections (*$p < 0.05$; **$p < 0.01$). BiP = binding immunoglobulin protein; CHOP = C/EBP homologous protein; ER = endoplasmic reticulum; GLT8D1 = glycosyltransferase 8 domain-containing protein 1; MPZ = myelin protein zero; RT-qPCR = real-time quantitative PCR; SEM = standard error of the mean; sXBP1 = spliced X-box–binding protein 1; WT = wild-type.

of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines,[18] *GLT8D1* p.I290M matches the PS3, PM2, PP3, and PP4 criteria and is classified as a likely pathogenic variant.

The GLT8D1 is a member of the glycosyltransferase family 8 and catalyzes transglycosylation reactions, where the monosaccharide component of a high-energy nucleotide sugar donor is transferred to an acceptor.[7] Aberrant glycosylation has been implicated in neurodegeneration through 2 main glycosyltransferase-related mechanisms: ganglioside synthesis and addition of O-linked β-N-acetylglucosamine to proteins (O-GlcNAcylation).[7] Gangliosides can modulate cell signaling processes, and O-GlcNAcylation is vital for axonal and synaptic function.[7] Defective glycosyltransferase-related mechanisms have been showed in animal models and patients of neurodegenerative diseases, such as Parkinson disease, Huntington disease, Alzheimer disease, and ALS.[7] In this study, we demonstrated impaired glycosyltransferase function of the I290M GLT8D1 variant protein, which is consistent with previous observations in other disease-associated GLT8D1 variants.[6] Notably, we observed mislocalization of I290M GLT8D1 to ER as well as activation of ER stress and UPR. These phenomena were not shown in the cells expressing R92C GLT8D1. ER stress has been widely implicated in ALS.[3] Our findings suggest that the *GLT8D1* p.I290M variation has more than 1 mechanism disturbing cellular homeostasis, including impairment of glycosyltransferase activity and disruption of ER function, which contribute to both loss-of-function and toxic gain-of-function effects on the disease pathogenesis.

The clinical features of ALS associated with *GLT8D1* variations are still not fully elucidated. *GLT8D1* variations may be associated with a typical ALS phenotype with highly variable age at disease onset and survival. In our study, the patient with the p.I290M variation had a spinal-onset ALS with disease onset at age 60 years and a survival of 6 years. Clear information of her affected younger brother was unavailable except knowing he is having an earlier disease onset and dying at age 30 years. In the study by Cooper-Knock et al.,[6] all the patients with ALS with a *GLT8D1* variation had a spinal or bulbar-onset disease with onset ages ranging from 33 to 66 years and disease survivals ranging from 6 to 101 months. In another study, the Chinese male patient harboring a heterozygous *GLT8D1* p.G78A variation had a right upper limb-onset ALS since age 44 years.[8] He was still alive in last evaluation at 10 months after disease onset. More studies are needed to conclude the phenotypic characteristics of *GLT8D1*-associated ALS.

The prevalence of *GLT8D1* variations in ALS appears to be low. The British study identified *GLT8D1* variations in 7 patients from 103 familial and young sporadic ALS cases, including 34 familial patients with ALS in whom a genetic cause had not been identified after screening for ALS-associated variations in SOD1, C9ORF72, TARDBP, and FUS.[6] In our study, the *GLT8D1* p.I290M variation was identified in 1 of the 477 unrelated patients with ALS (0.2%, 1/477). In another Chinese study, only 1 likely pathogenic variant of *GLT8D1*, p.G78A, was found in 1 single patient with familial ALS after screening 977 patients with sporadic ALS and 47 patients with familial ALS (0.1%, 1/1024).[8] Another 2 Chinese studies and 1 Australian study failed to identify any pathogenic variation after screening 512, 539 and 699 patients with ALS, respectively.[9-11] These findings suggest that *GLT8D1* variations are not a common cause of ALS.

All the *GLT8D1* pathogenic variations identified in patients with ALS previously are located within exon 4, including p.I70T, p.G78A, p.G78W, p.A82E, p.I87N, and p.R92C.[6,8] However, the *GLT8D1* p.I290M variation identified in this study resides in exon 9, demonstrating that variations in other exons of *GLT8D1* could also contribute to ALS. Of interest, a *GLT8D1* variant of unknown significance reported in a Chinese patient with familial ALS, p.V291I, is just located next to the p.I290M variation.[8]

In conclusion, we identified a novel *GLT8D1* variation, p.I290M, in 1 (0.2%) of 477 unrelated Taiwanese patients with ALS and demonstrated that this variation may lead to impaired GLT8D1 glycosyltransferase activity and aberrantly activation of ER stress. This study broadens the spectrum of *GLT8D1* variations as well as highlights the role of *GLT8D1* in ALS pathogenesis.

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Disclosure

The authors report no disclosure relevant to the article. Go to Neurology.org/NG for full disclosure.

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Ting-Yi Shen, BS	Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan	Major role in the acquisition of data
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Kon-Ping Lin, MD	Department of Neurology, Taipei Veterans General Hospital, Taipei, Taiwan; Department of Neurology, National Yang Ming Chao Tung University School of Medicine, Taipei, Taiwan	Major role in the acquisition of data
Yi-Chu Liao, MD, PhD	Department of Neurology, Taipei Veterans General Hospital, Taipei, Taiwan; Department of Neurology, National Yang Ming Chao Tung University School of Medicine, Taipei, Taiwan; Brain Research Center, National Yang Ming Chao Tung University, Taipei, Taiwan	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Yi-Chung Lee, MD, PhD	Department of Neurology, Taipei Veterans General Hospital, Taipei, Taiwan; Department of Neurology, National Yang Ming Chao Tung University School of Medicine, Taipei, Taiwan; Brain Research Center, National Yang Ming Chao Tung University, Taipei, Taiwan	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

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