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Two rare variants of the *ANXA11* gene identified in Chinese patients with amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder. A recent study has identified mutations in the *ANXA11* gene (encoding the calcium-binding protein annexin A11) associated with ALS. Mutation screening of *ANXA11* protein-coding exons was performed in a Chinese cohort of 434 patients with sporadic ALS and 50 index patients with familial ALS. Polymerase chain reaction and Sanger sequencing were used for mutation detection. We failed to discover an N-terminal mutation, which was common in the Caucasian cohort. We revealed two rare heterozygous missense variants, c.878C>T (p.A293V) and c.921C>G (p.I307M), which are absent from the population databases and non-neurological controls. They are both located in the conserved annexin domain. The carriers of the mutation exhibited the classical ALS phenotype without cognitive impairment. Our results suggested that further functional studies for these variants are required to support the pathogenicity.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease characterized by selective degeneration of upper and lower motor neurons. Approximately 10% of patients with ALS have a positive family history, whereas others have sporadic ALS (Ajroud-Driss and Siddique, 2015). More than 20 disease-causing genes have been detected through segregation analysis and burden analysis, of which *C9orf72*, *SOD1*, *FUS*, and *TARDBP* remain the most common genetic cause of both familial and seemingly sporadic ALS (Smith et al., 2017). Investigation of the genetic backgrounds of patients with ALS provides better understanding of molecular mechanisms of the disease.

The annexin A11 gene (*ANXA11*) encodes a calcium-dependent phospholipid-binding protein, which has the longest N-terminal in the annexin family (Lecona et al., 2003). The N-terminal (196 residues) contains a binding site of calcyclin (CACY, encoded by S100A6 gene). The C-terminal of the annexin A11 protein consists of four highly conserved annexin domains, which, with facilitation by Ca^{2+}, form an α-helical disk that connects phospholipids in cell membranes. The physiological role of the protein has not been fully understood, but evidence has shown that annexin A11 is associated with autoimmune disorders, such as sarcoidosis, systemic lupus erythematosus (Hofmann et al., 2008; Wang et al., 2014), and ovarian cancer recurrence (Song et al., 2009).

The *ANXA11* gene mutation (p.D40G) was recently identified in a proband of European familial ALS by whole exome sequencing analysis (Smith et al., 2017). The *ANXA11* mutations exhibited an autosomal dominant pattern, and the pathogenic role was supported on both pathological and genetic levels. The whole exome sequencing research revealed that disease-relevant mutations were enriched in the N-terminal (including p.D40G, p.G38R, and p.G175R); it has been proven that the mutations could disturb the binding site (residues 50–62) of CACY. Moreover, a previous study has shown that S100A6 is overexpressed within astrocytes from both an ALS mouse model and human patients. Mutations in the conserved *ANXA11* C-terminal can also be pathogenic (such as p.R235Q and p.R346C), which is supported by intracellular inclusions in human embryonic kidney 293 cells with p.R235Q mutation.

However, ethnic differences in ALS exist on both clinical and genetic levels. Chinese patients with ALS exhibit an earlier age of disease onset, better prognosis, and an extremely low frequency of *C9orf72* gene (Shahrizaila et al., 2016). Our aim was to investigate the frequency and mutations in the *ANXA11* gene in a Chinese cohort of patients with ALS.

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2. Materials and methods

2.1. Participants

A total of 434 patients with sporadic ALS and 50 index patients with familial ALS were recruited in this study. All patients were of Han ethnicity and were registered at the Neurological Department of Peking University Third Hospital from 2007 to 2014. Research participants were all evaluated using a standard protocol, which included a complete history, neurological examination, neuropsychological assessment, and biochemical studies. All patients met the revised El Escorial criteria of clinically definite, probable, or laboratory-supported probable ALS (Brooks et al., 2000).

In addition, patients were participating in long-term follow-up examinations every 3 months. We also screened for *SOD1*, *TARDBP*, *FUS*, *C9orf72*, *MATR3*, *TUBA4A*, *CHCHD10*, and *UBQLN2* genes in the same cohort before study enrollment (Li et al., 2016; Liu et al., 2013; Xu et al., 2016).

A Chinese Han ethnic control cohort of 370 non-neurologic individuals was also included in the study. The cohort consisted of volunteers of the hospital and spouses of patients.

This study was approved by the institutional ethics committee of Peking University Third Hospital (IRB00006761). The study group obtained written informed consent for genetic and clinical research from each patient before they participated in the study.

2.2. DNA sequencing

Genomic DNA was extracted from peripheral blood leukocytes using standard protocols (QIAGEN, Valencia, California, USA). A total of 11 pairs of primers were designed for polymerase chain reaction (PCR) to amplify the 14 protein-coding exons (exon 1 does not contain coding region) and intron-exon boundaries of *ANXA11* gene (GenBank NM_001157.2). The primers were designed using Primer3Plus (http://www.primer3plus.com) and are listed in Supplementary Table 1.

PCR was performed in a total volume of 25 μL, which consisted of 20 ng of genomic DNA, 10 pmol of each primer, 12.5 μL of 2× Taq PCR Master Mix (Tsingke Biotechnology Co, Ltd, Beijing, China) and 9.5 μL of deionized water. The thermocycling conditions were as follows: initial denaturation at 98 °C for 2 minutes; 30 cycles at 98 °C for 10 seconds, 65 °C for 15 seconds, and extension at 72 °C for 15 seconds; and a final extension at 72 °C for 5 minutes (the primer and annealing temperature was listed in supplementary table 1). The PCR products were purified and sequenced at Tsingke Biotechnology Co, Ltd. Each identified mutation was confirmed by both forward and reverse sequencing, and all mutated sequences were reamplified and resequenced.

2.3. Bioinformatics analysis

The sequencing results were analyzed using DNAStar Lasergene 7.1 software. The variants were identified in the Short Genetic Variations Database (dbSNP build 151, http://www.ncbi.nlm.nih.gov/SNP/), the NHLBI Exome Variant Server (http://evs.gs.washington.edu/EVS/), the 1000 Genomes Project, and the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org). The PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), MutationTaster (http://www.mutationtaster.org), and PROVEAN (http://provean.jcvi.org/) programs were used to evaluate the deleterious effects of mutant annexin A11 protein. We measured the evolutionary conservation using PhyloP and PhastCons (http://compgen.bscb.cornell.edu/phast/) software.

3. Results

In the sporadic ALS cohort, the male:female ratio was 1.86:1, the mean onset (age ± standard deviation [SD]) was 53.25 ± 11.52 years. In familial cases, the male:female ratio was 1.63:1, the mean onset (age ± SD) was 48.66 ± 12.56 years.

3.1. Genetic analysis

Sequence analysis of *ANXA11* revealed two single-nucleotide variants in exon 8. These missense heterozygous variants were c.878C>T (p.A293V) and c.921C>G (p.I307M) (Fig. 1). These two sites were wild type in 370 controls, and both were absent from the public population databases, including Short Genetic Variations Database, NHLBI Exome Variant Server, the 1000 Genomes Project, and ExAC. Our previous screens for common ALS genes also excluded mutations in *SOD1*, *TARDBP*, *FUS*, *C9orf72*, *SQSTM1*, *MATR3*, *TUBA4A*, *DCTN1*, *CHCHD10*, and UBQLN2 gene in these two variant carriers.

Supported by PhyloP and PhastCons software, both p.A293V and p.I307M are located in a highly conserved annexin domain and did not affect splicing. Both variants were predicted to have a deleterious effect by the PolyPhen-2 and MutationTaster programs, whereas PROVEAN predicted them to be neutral. The software prediction and scores of these sites are shown in Supplementary Table 2.

We also detected a c.107C>G (p.P36R) variant and 8 other nonsynonymous variants (all listed in Supplementary Table 2), but they were either present in the control cohort or occurred in

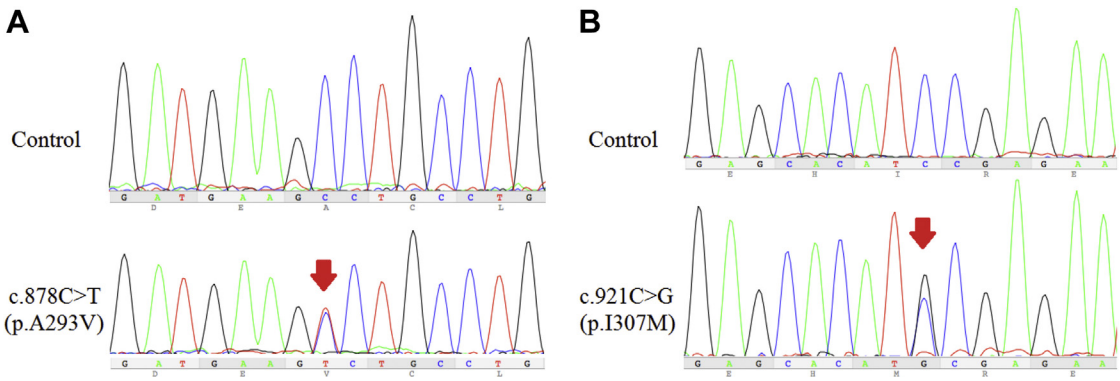

Fig. 1. Novel rare missense variants of *ANXA11* identified in patients with sporadic ALS. (A) Sequence chromatograms of polymerase chain reaction (PCR) products show the heterozygous g.878C>T (p.A293V) variant compared with wild-type control. (B) Sequence chromatograms of PCR products show the heterozygous g.921C>G (p.I307M) variant compared with wild-type control. Abbreviation: ALS, amyotrophic lateral sclerosis.

Table 1
Clinical characteristics of amyotrophic lateral sclerosis patients with *ANXA11* gene rare variants

Mutation	Gender	Onset age (y)	Site of onset	Diagnosis delay (mo)	Duration of disease (mo)	First ALS-FRS	FTLD symptom
p.A293V	Female	53	left hand	13	35	37	No
p.I307M	Male	55	bulbar	17	46	47	No

Key: ALS-FRS, amyotrophic lateral sclerosis functional rating scale; FTLD, frontotemporal lobar degeneration.

population databases. Thus, we suggest that these variants are likely to be benign. We also assessed the frequency of the p.R230C risk allele (rs1049550), which is associated with sarcoidosis, in our cohorts; there was no difference between the frequency in the ALS cohort (minor allele frequency [MAF] = 0.6808) or in the ExAC East Asian population (MAF = 0.6845, listed in Supplementary Table 2). The p value was 0.816 (by Pearson χ^2 test).

3.2. Clinical features

The *ANXA11* p.A293V mutation carrier was a female, who presented symptoms of weakness in the right hand in January 2013 at 53 years of age. After 7 months, all extremities were involved, and she also developed bulbar symptoms. Her family history was negative. On neurologic examination, although marked atrophy of extremities was noticed, deep tendon reflex was preserved. The neurophysiological study revealed diffuse neurogenic changes. A conclusion of definite ALS was established 13 months after disease onset. ALS functional rating scale score at the first visit was 37/48. 35 months after disease onset, the patient received tracheostomy and mechanical ventilation due to respiratory failure (summarized in Table 1).

The p.I307M mutation carrier was a male with disease onset at 55 years of age. He presented with neck weakness and dysarthria in August 2012. The patient reported no limb weakness, but electromyography revealed neurologic changes in both upper limb and rectus abdominis muscle. He denied any family history, and the neuropsychological testing was unremarkable. His diagnosis was lab-supported ALS, and the diagnosis delay was 17 months. The ALS functional rating scale score was 47/48 at the first visit. The patient died as a result of respiratory failure in June 2016; the survival time was 46 months (summarized in Table 1). Unfortunately, the DNA samples of family members for these two cases were unavailable.

4. Discussion

The present study investigated the recently discovered ALS-related gene *ANXA11* in a Chinese Han ethnic cohort. Sequencing was performed in 434 sporadic patients and 50 index patients of familial ALS.

Annexin A11 belongs to group A annexins, which are widely expressed in vertebrates. Most members of the annexin family share a four-homologous domain, and α-helix is the mainly secondary structure of the tetrad core (Gerke and Moss, 2002). Annexin A11 has the longest N-terminal in the annexin family, which contains a calcium binding site and contributes significantly to the overall structure of annexin. Annexin A11 induces vesicle aggregation, regulates exocytosis, and is involved in cell cycle progression (Lecona et al., 2003).

We identified two rare missense variants, p.A293V and p.I307M, in the *ANXA11* gene in patients with ALS. These two variants were absent from the controls cohort and public population databases, which included more than 65,000 samples. Software analysis suggests that the amino acids were evolutionarily conserved, and both are located in exon 8 (Fig. 2), which contains one of the homologous domain and forms the second α-helix of the core. Predictions of the functional impact of these mutations remain controversial. We did not have the parental genotype data of these carriers. Thus, we suggest further biological studies are necessary.

We failed to identify the hotspot mutation p.D40G reported in Caucasian cohorts in our Chinese cohort of patients with ALS. It is noteworthy that the haplotype analysis revealed the p.D40G mutation carriers in two British families shared a common European founder. This must be considered when interpreting the prevalence of *ANXA11* mutation in European familial cases. The *ANXA11* p.D40G affected carriers presented with a late disease onset (72 years on average) (Smith et al., 2017). In the present study, compared with the Chinese patients with ALS cohort (mean disease onset age of 50 years, median overall survival time of 71 months) (Chen et al., 2015), the carriers of the p.A293V and p.I307M variants did not exhibit late-onset disease but had rapid disease progression (35–46 months vs. 71 months). We failed to discover *ANXA11* mutations in familial ALS probands, but the sample size of familial cases was relatively small to give a positive result. Increasing the sample size in future studies will be helpful.

The recent study of the *ANXA11* gene in non-Caucasian patients with ALS supported the pathogenicity of p.D40G mutation (Zhang et al., 2018); the mutation was found once in a sporadic ALS case in 383 patients with ALS in that study. If we combine the results with ours, the p.D40G mutation in Chinese patients with ALS has a relatively low frequency of 0.12% (1/867), which suggests that p.D40G mutation is a rare cause of ALS in the Chinese population. Zhang et al have meanwhile found another seven variants in a total

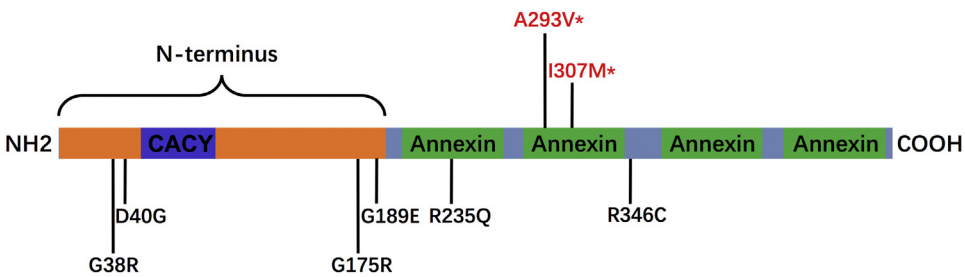

Fig. 2. The genetic location of mutations in the *ANXA11* gene. Schematic representation of the annexin A11 protein and functional domains: N-terminal (residues 1-196, including calcyclin binding site) and four annexin domains. Reported ALS-related mutations in Europeans are displayed in black, and rare variants detected in the present study are displayed in red with an asterisk. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

of 383 patients with ALS. The present study was conducted in the same racial background, but we reported only two speculated disease-related variants in our patients with ALS. It is because we applied a strict cutoff value of MAF in the ExAC database to predict the pathogenicity of variants (0 vs. 0.005%). We interpreted these variants which appeared once or more in the population databases as variants of unknown significance or benign variants. However, the c.107C>G (p.P36R) variants were found once in the present ALS cohort and three cases in the cohort of Zhang et al., 2018. Although it is presented once in the ExAC East Asian population (MAF = 0.00012), the p.P36R mutation has a higher frequency of 0.46% (4/867) in Chinese patients with ALS. The p.P36R variant is located in the CACY binding site in the N-terminal of annexin A11 near the p.G38R and p.D40G mutation, although the allele in same site c.107C>T (p.P36H) variant appeared more frequently in the ExAC East Asian population database (MAF = 0.00049). Whether the variant is pathogenic is still an open question.

In conclusion, we screened the ALS-related gene *ANXA11* in a Chinese cohort of 434 sporadic and 50 patients with familial ALS. We did not find the hotspot mutation discovered in Caucasian cohorts. We observed 2 rare variants, p.A293V and p.I307M, in the sporadic ALS cohort. The carriers exhibited rapid disease progression but normal disease onset age. Our results were insufficient to conclude the pathogenicity of *ANXA11* mutation, but the comparison of genetic variants in different racial backgrounds is still an important way to understand the disease biology and will contribute to further functional studies.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018.09.020.

References

Ajroud-Driss, S., Siddique, T., 2015. Sporadic and hereditary amyotrophic lateral sclerosis (ALS). Biochim. Biophys. Acta 1852, 679–684.

Brooks, B.R., Miller, R.G., Swash, M., Munsat, T.L., World Federation of Neurology Research Group on Motor Neuron, D., 2000. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Other Motor Neuron Disord. 1, 293–299.

Chen, L., Zhang, B., Chen, R., Tang, L., Liu, R., Yang, Y., Yang, Y., Liu, X., Ye, S., Zhan, S., Fan, D., 2015. Natural history and clinical features of sporadic amyotrophic lateral sclerosis in China. J. Neurol. Neurosurg. Psychiatry 86, 1075–1081.

Gerke, V., Moss, S.E., 2002. Annexins: from structure to function. Physiol. Rev. 82, 331–371.

Hofmann, S., Franke, A., Fischer, A., Jacobs, G., Nothnagel, M., Gaede, K.I., Schurmann, M., Muller-Quernheim, J., Krawczak, M., Rosenstiel, P., Schreiber, S., 2008. Genome-wide association study identifies ANXA11 as a new susceptibility locus for sarcoidosis. Nat. Genet. 40, 1103–1106.

Lecona, E., Turnay, J., Olmo, N., Guzman-Aranguez, A., Morgan, R.O., Fernandez, M.P., Lizarbe, M.A., 2003. Structural and functional characterization of recombinant mouse annexin A11: influence of calcium binding. Biochem. J. 373 (Pt 2), 437–449.

Li, J., He, J., Tang, L., Chen, L., Xu, L.P., Ma, Y., Zhang, N., Fan, D.S., 2016. TUBA4A may not be a significant genetic factor in Chinese ALS patients. Amyotroph. Lateral Scler. Frontotemporal Degener. 17, 148–150.

Liu, R., Tang, L., Cai, B., Liu, X.L., Ye, S., Ma, Y., Zhang, H.G., Fan, D.S., 2013. C9orf72 repeat expansions are not detected in Chinese patients with familial ALS. Amyotroph. Lateral Scler. Frontotemporal Degener. 14, 630–631.

Shahrizaila, N., Sobue, G., Kuwabara, S., Kim, S.H., Birks, C., Fan, D.S., Bae, J.S., Hu, C.J., Gourie-Devi, M., Noto, Y., Shibuya, K., Goh, K.J., Kaji, R., Tsai, C.P., Cui, L., Talman, P., Henderson, R.D., Vucic, S., Kiernan, M.C., 2016. Amyotrophic lateral sclerosis and motor neuron syndromes in Asia. J. Neurol. Neurosurg. Psychiatry 87, 821–830.

Smith, B.N., Topp, S.D., Fallini, C., Shibata, H., Chen, H.J., Troakes, C., King, A., Ticozzi, N., Kenna, K.P., Soragia-Gkazi, A., Miller, J.W., Sato, A., Dias, D.M., Jeon, M., Vance, C., Wong, C.H., de Majo, M., Kattuah, W., Mitchell, J.C., Scotter, E.L., Parkin, N.W., Sapp, P.C., Nolan, M., Nestor, P.J., Simpson, M., Weale, M., Lek, M., Baas, F., Vianney de Jong, J.M., Ten Asbroek, A., Redondo, A.G., Esteban-Perez, J., Tiloca, C., Verde, F., Duga, S., Leigh, N., Pall, H., Morrison, K.E., Al-Chalabi, A., Shaw, P.J., Kirby, J., Turner, M.R., Talbot, K., Hardiman, O., Glass, J.D., De Belleroche, J., Maki, M., Moss, S.E., Miller, C., Gellera, C., Ratti, A., Al-Sarraj, S., Brown Jr., R.H., Silani, V., Landers, J.E., Shaw, C.E., 2017. Mutations in the vesicular trafficking protein annexin A11 are associated with amyotrophic lateral sclerosis. Sci. Transl. Med. 9, eaad9157.

Song, J., Shih Ie, M., Chan, D.W., Zhang, Z., 2009. Suppression of annexin A11 in ovarian cancer: implications in chemoresistance. Neoplasia 11, 605–614, 1 p following 14.

Wang, J., Guo, C., Liu, S., Qi, H., Yin, Y., Liang, R., Sun, M.Z., Greenaway, F.T., 2014. Annexin A11 in disease. Clin. Chim. Acta 431, 164–168.

Xu, L.P., Li, J., Tang, L., Zhang, N., Fan, D.S., 2016. MATR3 mutation analysis in a Chinese cohort with sporadic amyotrophic lateral sclerosis. Neurobiol. Aging 38, 218.e3–218.e4.

Zhang, K., Liu, Q., Liu, K., Shen, D., Tai, H., Shu, S., Ding, Q., Fu, H., Liu, S., Wang, Z., Li, X., Liu, M., Zhang, X., Cui, L., 2018. ANXA11 mutations prevail in Chinese ALS patients with and without cognitive dementia. Neurol. Genet. 4, e237.