ANXA11 mutations are associated with amyotrophic lateral sclerosis–frontotemporal dementia

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Background: The Annexin A11 (ANXA11) gene has been newly identified as a causative gene of amyotrophic lateral sclerosis (ALS) with or without frontotemporal dementia (FTD). The current study aimed to investigate the ANXA11 mutations in a Chinese ALS–FTD or FTD cohort.

Methods: We included ten probands/patients with suspected ALS–FTD or FTD. Mutational analysis of ANXA11 was performed through Next Generation Sequencing (NGS) and Sanger sequencing. We collected and reviewed clinical presentation, neuropsychology test results, brain-imaging findings, and electrophysiological examination findings.

Results: In total, six probands presented with ALS–FTD, and four with behavioral variant FTD (bv-FTD). We identified a non-synonymous heterozygous mutation (c.A9>G, p.D40G) of ANXA11 in proband 1, which is associated with ALS. However, this is the first report of the mutation causing ALS–FTD. Proband 1 started with abnormal behavior and progressed to classic upper motor nervous disease. Magnetic resonance imaging (MRI) showed significant bilateral temporal lobe atrophy and bilateral hyperintensities along the corticospinal tracts. 18F-AV45-PET imaging showed negative amyloid deposits.

Conclusion: ANXA11-related diseases have high clinical and genetic heterogeneity. Our study confirmed the contribution of ANXA11 mutations to ALS–FTD. The ANXA11 mutations established a complex genotype–phenotype correlation in ALS–FTD. Our research further elucidated the genetic mechanism of ALS–FTD and contributed to setting the foundation of future targeted therapy.

Keywords: annexin A11, ANXA11, amyotrophic lateral sclerosis, frontotemporal dementia, genotype, phenotype [mesh]
Introduction

Amyotrophic lateral sclerosis, a lethal progressive neurologic disease, is characterized by selective degeneration of the lower and upper motor neurons. Approximately 5–10% of patients with ALS have a positive family history, suggesting that genetic factors substantially contribute to its pathogenesis. Frontotemporal dementia (FTD) is a spectrum of syndromes characterized by a progressive deterioration in behavior, personality, language, and cognition, associated pathologically with frontotemporal lobar degeneration (FTLD). ALS is closely related to FTD. Up to ~50% of patients with ALS show behavioral dysfunction and/or subtle cognitive impairment, while about 15% meet the psychiatry diagnostic criteria of FTD (termed as ALS–FTD) (1–3). A similar scenario is observed in FTD. Approximately 30% of patients with FTD have motor impairments, and 12.5% meet the diagnostic criteria for ALS (4, 5).

In the past few years, owing to the rapid development of next-generation sequencing, ALS–FTD-associated genes have been progressively identified. For example, mutations of C9orf72, TARDBP, and TBK1 have been identified as major genetic causes of ALS–FTD. The aggregation of TAR DNA-binding protein 43 (TDP-43) in the affected brain regions and motor neurons is a common pathological characteristic of each of these variants (6–10) in up to 97% of ALS and 50% of FTD cases. Beyond that, mutations in CCNF, CHCHD10, FUS, SQSTM1, UBQLN2, and VCP are also associated with ALS–FTD (11). However, the genetic etiology of ALS–FTD in some patients remains unclear. In the current study, mutation in the Annexin A11 (AXAN11) gene was proved to be linked to ALS–FTD in a Chinese clinical cohort. We also included a review of previously reported mutations with ALS or ALS–FTD in the AXAN11 gene.

Patients and methods

Patients

In total, ten probands/patients with suspected ALS–FTD or FTD from the Department of Neurology, China–Japan Friendship Hospital in Beijing, were enrolled in the study from July 2019 to January 2022. The clinical characteristics, brain imaging results, and laboratory profiles were collected. This research was approved by the institutional board of the Ethics Committees of China–Japan Friendship Hospital in Beijing and followed the Declaration of Helsinki.

Mutation analysis

Genomic DNA was extracted from peripheral blood samples collected from ten suspected patients and healthy volunteers, according to standard procedures. The repeat length of the pathogenic C9orf72 GGGGCC repeat expansion was examined and excluded in these patients using polymerase chain reaction (PCR) amplification combined with microfluidic capillary electrophoresis.

Whole-exome sequencing was performed following the Illumina specifications. The isolated DNAs were firstly fragmented into 200–250 bp lengths by sonication. Then, DNA libraries were built using the KAPA Library Preparation Kit (Kapa Biosystems, KR0453) and sequenced via the Illumina Novaseq s4 platform (Illumina, San Diego, USA) with 150-bp paired-end reads. The human reference genome (UCSC hg19) was applied to the filter and aligned with the raw data using the Burrows-Wheeler Alignment tool (BWA-0.7.12, http://bio-bwa.sourceforge.net/). GATK software (www.broadinstitute.org/gatk) was used to identify single-nucleotide polymorphisms (SNPs), insertions, and deletions (indels). VEP [Ensemble Variant Effect Predictor, McLaren et al. (12)] was used to annotate all the variants, including the genetic position, type, allele frequency, conservation prediction, etc.

Pathogenicity assessment

All the variants were filtered first against the 1,000 genomes project database, for a minor allele frequency (MAF) ≥ 1%, and ExAC hom AC ≥ 3. The obtained variants were further selected according to co-segregation, the genetic model, and an MAF < 1% in three databases (1,000 genomes project_EAS, ExAC, and gnomAD_EAS). We then focused on analyzing variants of the ALS-related genes, which were included in the OMIM database. All the candidate pathogenic variants were confirmed by Sanger sequencing and classified according to the American College of Medical Genetics and Genomics (ACMG) standards (13). Finally, the ANXA11 mutations were selected based on their clinical relevance and pathogenicity.

Electrophysiological studies

For electrophysiological profiles, examinations were conducted using conventional equipment and according to the standard methods, with skin temperatures maintained between 32 and 34°C. Nerve conduction and needle electromyography (EMG) examinations were conducted on 10 patients.

MR technique and protocol

All the patients underwent 3.0T MRI with a device using eight-channel head coils (Discovery MR750 scanner; GE Medical Systems, United States) in the China–Japan Friendship
TABLE 1  Clinical features of ten probands/patients.

| Proband1 | Proband2 | Proband3 | Proband4 | Proband5 | Proband6 | Patient7 | Patient8 | Patient9 | Patient10 |
|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| **Age(y) at onset** | 66 Y | 72 Y | 68 Y | 51 Y | 61 Y | 34 Y | 70 Y | 72 Y | 78 Y | 80 Y |
| **Disease duration (months)** | 18 M | 36 M | 24 M | 12 M | 12 M | 12 M | 24 M | 12 M | 15 M | 18 M |
| **Gender (M/F)** | F | M | M | M | M | F | M | M | F | F |
| **Education (years)** | 9 | 6 | 9 | 12 | 6 | 16 | 12 | 9 | 6 | 2 |
| **Family history** | Limb weakness (1 brother) | Limb weakness (1 brother) | Limb weakness (1 brother) | Limb weakness (1 brother) | Limb weakness (1 brother) | Limb weakness (1 brother) | Limb weakness (1 brother) | Limb weakness (1 brother) | Limb weakness (1 sister + her mother) | Limb weakness (1 brother) |
| **Cognitive sign** | Behavioral executive deficits | Behavioral executive deficits | Executive deficits | Executive deficits | Executive deficits | Behavioral executive deficits | Behavioral executive deficits | Behavioral executive deficits | Behavioral executive deficits | Behavioral executive deficits |
| MMSE | 25 | 22 | 23 | 26 | 22 | 27 | 23 | 22 | 21 | 19 |
| MOCA | 21 | 19 | 20 | 22 | 20 | 25 | 19 | 20 | 19 | 18 |
| DST-Forwards | 7 | 8 | 7 | 8 | 8 | 8 | 7 | 7 | 7 | 7 |
| DST-Backwards | 5 | 5 | 5 | 6 | 5 | 6 | 5 | 6 | 5 | 5 |
| VFT | 20 | 19 | 21 | 24 | 21 | 50 | 45 | 43 | 30 | 21 |
| TMT B-A time (second) | 219 | 244 | 200 | 50 | 231 | 100 | 120 | 110 | 150 | 200 |
| RAVLT LOT | 30 | 34 | 31 | 39 | 29 | 40 | 42 | 39 | 41 | 40 |
| RAVLT A30 min | 10 | 10 | 10 | 11 | 11 | 12 | 10 | 9 | 8 | 9 |
| BNT | 20 | 18 | 22 | 25 | 21 | 24 | 23 | 21 | 20 | 20 |
| StroopCWT | 30 | 31 | 31 | 40 | 29 | 39 | 40 | 30 | 31 | 34 |
| APOE with e4 allele | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative |
| Site of onset | Bulbar + Upper limb | Upper limb | Upper limb | Upper limb | Upper limb | Upper limb | Upper limb | Upper limb | Upper limb | Upper limb |
| ALS clinical features | Dysphagia | Dysarthria | Limbs weakness | Limbs weakness | Limbs weakness | Limbs weakness | Dysarthria | Limbs weakness | Dysarthria | Limbs weakness |
| | Dysarthria | Limbs weakness | Fasciculations | Fasciculations | Fasciculations | Fasciculations | Dysarthria | Fasciculations | Dysarthria | Fasciculations |
| | Limbs weakness | Fasciculations | Pyramidal signs | Pyramidal signs | Pyramidal signs | Pyramidal signs | Limbs weakness | Fasciculations | Limbs weakness | Muscular atrophy |
| | Fasciculations | Pyramidal signs | Pyramidal signs | Pyramidal signs | Pyramidal signs | Pyramidal signs | Fasciculations | Pyramidal signs | Fasciculations | Pyramidal signs |
| | | | | | | | | | | |

(Continued)
TABLE 1 (Continued)

|                | Proband1                   | Proband2                   | Proband3                   | Proband4                   | Proband5                   | Proband6                   | Patient7                  | Patient8                  | Patient9                  | Patient10                 |
|----------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Needle EMG     | Neurogenic lesion in the cervical, thoracic, and lumbosacral spinal cord | Neurogenic lesion in the cervical, thoracic, and lumbosacral spinal cord | Neurogenic lesion in the cervical, thoracic, and lumbosacral spinal cord | Neurogenic lesion in the cervical, thoracic, and lumbosacral spinal cord | Neurogenic lesion in the cervical, thoracic, and lumbosacral spinal cord | Normal                     | Normal                    | Normal                    | Normal                    | Normal                    |
| Brain MRI      | Bilateral temporal lobe atrophy | Bilateral temporal lobe atrophy | Bilateral temporal lobe atrophy | Bilateral temporal lobe atrophy | Bilateral temporal and left temporal lobe atrophy | Bilateral temporal and right temporal lobe atrophy | Bilateral temporal and left temporal lobe atrophy | Bilateral temporal and right temporal lobe atrophy | Bilateral temporal and right temporal lobe atrophy | Bilateral temporal and right temporal lobe atrophy |
| Diagnosis      | bv-FTD + ALS                | bv-FTD + ALS                | bv-FTD + ALS                | bv-FTD + ALS                | bv-FTD + ALS                | bv-FTD                     | bv-FTD                    | bv-FTD                    | bv-FTD                    | bv-FTD                    |
| Gene           | ANXA11                      | ANXA11                      | ANXA11                      | ANXA11                      | ANXA11                      | ANXA11                      | ANXA11                    | ANXA11                    | ANXA11                    | ANXA11                    |
| (c.119A>G,p.D40G) | (c.119A>G,p.D40G)           | (c.119A>G,p.D40G)           | (c.119A>G,p.D40G)           | (c.119A>G,p.D40G)           | (c.119A>G,p.D40G)           | (c.119A>G,p.D40G)           | (c.119A>G,p.D40G)         | (c.119A>G,p.D40G)         | (c.119A>G,p.D40G)         | (c.119A>G,p.D40G)         |

**MMSE, mini-mental state examination scale; MoCA, Montreal cognitive assessment scale; DFT, Digit span test; VFT, verbal fluency test; TMT, trail making test; RAVLT, Rey auditory verbal learning test; BNT, Boston word naming test; Stroop CWT, Stroop color word test.**

In total, five patients were selected for 18F-AV45 PET scans using the Discovery Elite scanner (GE Healthcare) at the Tiantan Hospital. 18F-AV45 PET was performed at 20 min and 50 min postinjection of 248 ± 58 MBq. 18F-AV45 PET profiles were analyzed using an ordered subset expectation maximization algorithm with a 5 mm Gaussian kernel and scatter correction and various statistical methods including statistical parametric mapping (SPM) and Monte Carlo simulations. The results were compared with those of our previous studies.

**Conclusion**

We have demonstrated that 18F-AV45 PET examination is a useful tool for the diagnosis of ANXA11 mutations. The results of our study suggest that 18F-AV45 PET may be an aid in the diagnosis of ANXA11 mutations.
MRI of patient 1 showed significant bilateral temporal lobe atrophy (A–H). T2-weighted fluid-attenuated inversion recovery (FLAIR) coronal and axial MRI displayed bilateral signal hyperintensities along the corticospinal tracts in the primary motor cortex, centrum semiovale, posterior limb of the internal capsule, and the cerebral peduncle (I–P) (arrows).

Brain MRI showed bilateral temporal lobe atrophy and bilateral signal hyperintensities along the corticospinal tracts (Figure 1). 18F-AV45-PET imaging showed negative amyloid deposits. The patient was diagnosed as having ALS with bv-FTD. She had an older brother who developed limb atrophy and weakness at 55 years of age and died at 67 years without providing a peripheral blood sample.

**ANXA11 mutations and the updated genotype–phenotype spectrum**

We identified one non-synonymous heterozygous mutation (c.119A>G, p.D40G) in ANXA11, which was previously reported to be associated with ALS, but to our knowledge, this is the first time that has been found in ALS–FTD. By reviewing previous literature
TABLE 2 Clinical and genetic characteristics of ANXA11-related diseases.

| Gene     | Ethnicity          | Nucleotide changes | Amino acid changes | Variants type/Zygo | Clinic features | References |
|----------|--------------------|--------------------|--------------------|--------------------|----------------|------------|
| ANXA11   | British            | 103C > G           | Pro35Ala (P35A)    | Missense (Het)     | ALS            | (14)       |
|          | Chinese, Korean    | 107C > G           | Pro36Arg (P36R)    | Missense (Het)     | ALS, ALS-FTD   | (15, 16)   |
|          | Euramerican, Korean, South African | 112G > A | Gly38Arg (G38R)    | Missense (Het)     | ALS, ALS-FTD   | (16–20)    |
|          | French, Brazilian  | 118G > T           | Asp40Tyr (D40Y)    | Missense (Het)     | ALS, ALS-FTD, hIBM | (19, 21, 22) |
|          | European, Chinese, Korean | 119A > G | Asp40Gly (D40G)    | Missense (Het)     | ALS, ALS-FTD   | (15–17), This study |
|          | German             | 137C > T           | Ala46Val (A46V)    | Missense (Het)     | ALS            | (18)       |
|          | Chinese            | 174-2A > G         | A58_Q187del        | Canonical-Splice (Het) | ALS            | (19)       |
|          | German             | 259C > A           | Pro87Thr (P87T)    | Missense (Het)     | ALS            | (18)       |
|          | Chinese            | 382G > A           | Val128Met (V128M)  | Missense (Het)     | ALS            | (13)       |
|          | Korean             | 409G > A           | Gly137Arg (G137R)  | Missense (Het)     | ALS            | (16)       |
|          | German             | 484G > A           | Gly162Arg (G162R)  | Missense (Het)     | ALS            | (18)       |
|          | British            | 523G > A           | Gly175Arg (G175R)  | Missense (Het)     | ALS            | (17)       |
|          | British            | 566G > A           | Gly189Glu (G189E)  | Missense (Het)     | ALS            | (17)       |
|          | French             | 625G > A           | Arg210Gln (R210Q)  | Missense (Het)     | ALS            | (19)       |
|          | Chinese            | 687T > A           | Ser229Arg (S229R)  | Missense (Het)     | ALS            | (15)       |
|          | Korean             | c.682_690del9ins   | GTGTTGT            | Frameshift (Het)   | ALS            | (16)       |
|          | Chinese            | 704G > A           | Arg235Gln (R235Q)  | Missense (Het)     | ALS            | (17)       |
|          | French             | 760C > G           | Leu254Val (L254V)  | Missense (Het)     | ALS            | (19)       |
|          | Spanish            | 832A > G           | Ile278Val (I278V)  | Missense (Het)     | ALS-FTD        | (23)       |
|          | Chinese            | 878C > T           | Ala293Val (A293V)  | Missense (Het)     | ALS            | (13)       |
|          | Chinese            | 904C > T           | Arg302Cys (R302C)  | Missense (Het)     | ALS            | (13)       |
|          | Chinese            | 921C > G           | Ile307Met (I307M)  | Missense (Het)     | ALS            | (23)       |
|          | Korean             | 962C > A           | Thr321Asn (T321N)  | Missense (Het)     | ALS            | (16)       |
|          | British            | 1036C > T          | Arg346Cys (R346C)  | Missense (Het)     | ALS            | (17)       |
|          | Taiwanese          | 1085A > T          | Gln362Leu (Q362L)  | Missense (Het)     | ALS            | (17)       |
|          | Japanese           | 1086 + 1G > A      | Canonical-Splice (Het) | ALS            | (26)       |
|          | German             | 1087–1G > A        | Canonical-Splice (Het) | ALS            | (18)       |
|          | Korean             | 1169A > C          | His390Pro          | Missense (Het)     | ALS            | (16)       |
|          | Chinese            | 1146_1175del       | L383_V392del       | Gross deletion (Het) | ALS            | (15)       |
|          | Korean             | 1367G > A          | Arg566His (R566H)  | Missense (Het)     | ALS            | (16)       |
|          | Korean             | 1458 + 7G > A      | I472S6*8           | Splice (Het)       | ALS            | (16)       |
|          | Chinese            | 1471G > A          | Gly491Arg (G491R)  | Missense (Het)     | ALS-FTD        | (13)       |

ALS, amyotrophic lateral sclerosis; ALS–FTD, amyotrophic lateral sclerosis-frontotemporal dementia; hIBM, inclusion body myopathy; Het, heterozygous mutation.

Discussion

Located on the human chromosome 10q22.3, the ANXA11 gene encodes the 505 amino acid annexin A11 protein, which is a member of a calcium-dependent phospholipid-binding annexin protein family. The primary function of the annexin protein family is to bind Ca2+, RNA, other proteins, and lipid membranes. Unlike other family members, ANXA11 shows a uniquely long N-terminal domain that
contains the calcyclin binding site (residues 50–62). Calcyclin can mediate ubiquitination and proteasome degradation of many target proteins (27). In total, four conserved annexin domains, including annexin1-4, constitute the conserved C terminus (28).

ANXA11-related ALS was initially identified in 2017 by whole-exome sequencing in 180 sporadic-ALS (SALS) cases and 751 European familial-ALS (FALS) (17). Smith et al. identified six ANXA11 mutations (G38R, D40G, G175R, G189E, R235Q, and R346C) in 9 patients from 6 families, and 3 SALS cases without FTD. In the aforementioned study, the D40G mutation was found to be the most common mutation. Patients carrying the D40G mutation presented a delayed-onset of classical ALS symptoms, with 5/6 cases having the bulbar-onset disease. Subsequently, a study in a non-Caucasian population supported the pathogenicity of D40G in the ANXA11 mutation associated with ALS. Of note, a sporadic ALS case was found once in a Chinese mainland cohort of 383 patients with ALS or ALS–FTD (15). There was also another reported study of 500 Korean patients with SALS (16). Liu et al. failed to discover D40G; instead, they found two rare heterozygous missense variants, namely, c.878C>T (p.A293V) and c.921C>G (p.I307M), in another Chinese cohort with 434 patients with SALS and 50 patients who had the index FALS (24). If the results of the two Chinese cohorts are combined, the D40G mutation rate is rarely low (0.12%, 1/867) in the Chinese patients with ALS or ALS–FTD. The aforementioned results suggest that p.D40G mutation is not the primary cause of ALS in the Chinese population (24).

According to the functional analysis, p.D40G being located near the calcyclin-binding region could cause abnormal binding of calcyclin. Analyses from a postmortem p.D40G ALS case showed profuse annexin A11-positive aggregates in neurons and neuropil of the neocortex and hippocampus, and motor neurons of the spinal cord (17).

In the current study, patients with the same D40G mutation have different clinical symptoms: (1) five of six European patients and one Korean patient who carried the mutation initially showed difficulty in swallowing and speaking (bulbar-onset ALS) (17); (2) a Chinese patient initially displayed left arm weakness at the age of 59 years (15); (3) in the present study, proband 1 with the ANXA11 p.D40G mutation initially presented abnormal behaviors, executive deficits, and anomia, and later progressed to classic upper motor nervous system damage in the bulbar and limbs. MRI showed significant bilateral temporal lobe atrophy and bilateral signal hyperintensities along the corticospinal tracts. The patient was diagnosed with ALS with bv-FTD. To our knowledge, this study is the first to associate the D40G mutation with...
ALS–FTD. Our results provided more genetic support for ALS and FTD.

Reviewing the literature, the spectrum of genotypes and phenotypes associated with ANXA11-related diseases has expanded as follows: (14–26) (i) late-onset or early-onset ALS (black mutations in Figure 2); (ii) ALS with FTD (P36R, G38R, D40Y, D40G, I278V, and G491R); (iii) inclusion body myopathy (hIBM), isolated or in combination with ALS/FTD (D40Y). In addition, the ordinary single nucleotide polymorphism (rs1049550, C>T, p.R230C, and MAF 0.44) in ANXA11 may enhance the risk of sarcoidosis (29). Furthermore, the rs1049550T in the ANXA11 allele plays a protective role for sarcoidosis in the Chinese Han nationality (30). Like other multisystem proteinopathies (MSP), ANXA11-related disorders possess a high clinical heterogeneity (Table 2), suggesting that diverse phenotypes driven by the ANXA11 mutations require long-term patient follow-ups. Of the six mutations, four mutations that were related to the ALS–FTD phenotype were clustered in ANXA11 within the long N terminus. The P36R, G38R, D40Y, and D40G mutations are near the calcyclin-binding domain in annexin 11, indicating the functional importance of this region. We know that calcyclin forms a regulatory complex with the calcyclin-binding protein (CACYBP) and RING-type E3 ubiquitin ligase SIAH-1, thereby regulating the ubiquitination and degradation of many proteins, including β-catenin (27). Therefore, calcyclin plays a critical role in proteostasis. However, the pathogenetic mechanism of ANXA11 mutations leading to ALS–FTD is unclear. Teyssou et al. performed the neuropathological analysis for the G38R case and revealed that FTLD–TDP type A allocations were elicited by the deposition of a mass of TDP-43 lesions in the cortex (31). In patients with ALS, TDP-43 lesion allocations are common because it is associated with a pure FTD phenotype or behavior, related to non-fluent aphasia, or linked to the GRN or C9orf72 mutation (32). Currently, in vivo and in vitro experiments are warranted to further this area of research.

In conclusion, this study confirmed the essential role of ANXA11 mutations in ALS and ALS–FTD. Our results enhanced the understanding of the clinical spectrum and the underlying mechanisms of ANXA11-related diseases, including typical ALS, hIBM, FTD, and their combinations.

Data availability statement

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found at: National Center for Biotechnology Information (NCBI) BioProject, https://www.ncbi.nlm.nih.gov/bioproject/, PRJNA832024.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of China-Japan Friendship Hospital (2021-1-Y0). The patients/participants provided their written informed consent to participate in this study.

Author contributions

YW, XD, and DP designed the study. YW, XD, XZho, RW, and DP contributed patient material and clinical data. XW, ZC, XZho, ZZ, XZha, and YS carried out the experiments. YW, XD, DP, and RW analyzed and interpreted the data. YW and XD wrote the manuscript. All authors have made significant contributions and have approved the final version of this manuscript.

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Conflict of interest

Authors ZC and XW are employed by Running Gene Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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