Identification of a novel de novo pathogenic variant in GFAP in an Iranian family with Alexander disease by whole-exome sequencing

Katayoun Heshmatzad1, Niloofar Naderi1, Tannaz Masoumi1, Hamidreza Pouraliakbar2 and Samira Kalayinia1*

Abstract

Background: Alexander disease (AxD) is a rare leukodystrophy with an autosomal dominant inheritance mode. Variants in GFAP lead to this disorder and it is classified into three distinguishable subgroups: infantile, juvenile, and adult-onset types.

Objective: The aim of this study is to report a novel variant causing AxD and collect all the associated variants with juvenile and adult-onset as well.

Methods: We report a 2-year-old female with infantile AxD. All relevant clinical and genetic data were evaluated. Search strategy for all AxD types was performed on PubMed. The extracted data include total recruited patients, number of patients carrying a GFAP variant, nucleotide and protein change, zygosity and all the clinical symptoms.

Results: A novel de novo variant c.217A > G: p. Met73Val was found in our case by whole-exome sequencing. In silico analysis categorized this variant as pathogenic. Totally 377 patients clinically diagnosed with juvenile or adult-onset forms were recruited in these articles, among them 212 patients were affected with juvenile or adult-onset form carrier of an alteration in GFAP. A total of 98 variants were collected. Among these variants c.262C > T 11/212 (5.18%), c.1246C > T 9/212 (4.24%), c.827G > T 8/212 (3.77%), c.232G > A 6/212 (2.83%) account for the majority of reported variants.

Conclusion: This study highlighted the role of genetic in AxD diagnosing. It also helps to provide more information in order to expand the genetic spectrum of Iranian patients with AxD. Our literature review is beneficial in defining a better genotype–phenotype correlation of AxD disorder.

Keywords: Infantile Alexander disease, GFAP, Leukodystrophy, Whole-exome sequencing, Genetics, In silico analysis

Introduction

Alexander disease (AxD) (OMIM #203450) is a rare leukodystrophy first described in 1949 with usually infantile manifestation. The exact prevalence of AxD is not known, however a Japanese investigation estimated an incidence of 1 person in 2.7 million. This disorder belongs to a group of neurological diseases denoted as leukodystrophies affecting the central nervous system (CNS) white matter and characterized by myelin sheath defects or abnormal development of myelin sheath [1, 2]. According to age of onset, AxD is classified in to three subgroups naming infantile, juvenile and adult forms [3]. Patients affected with infantile AxD present various symptoms
such as seizures, megalencephaly, developmental delay, progressive deterioration and increased neonatal patients severity within first two years after birth [4]. Juvenile form with the age of onset (2–14 years of age) is characterized by symptoms including ataxia, hyperreflexia, bulbar symptoms. Juvenile form has milder progression and preserved cognitive and motor function comparing to infantile form. Adult AxD patients have more similarities to the juvenile form and manifest mainly spastic paraparesis, palatal myoclonus, bulbar symptoms and ataxia [5]. AxD is usually diagnosed based on the results of CT and MRI characteristic appearances—reference. Frontal predominance involvement, hindbrain involvement, medulla oblongata and cervical spinal cord atrophy are indicators of younger patients and patients with later onset, respectively [6–8]. This autosomal dominant disorder is usually the consequence of defects in GFAP gene [9]. Sporadic cases should be mentioned briefly GFAP is located within chromosome 17q21 consists of nine exons spreading 9.8 kb length encoding a 432 amino acid protein. This protein belongs to intermediate filament proteins and has considerable and key roles in astrocytes morphology and motility regulation and astrocytes and oligodendrocytes interaction. The exact and precise mechanism through which GFAP function is not completely understood, however, it is believed that gain of function mutations in GFAP affects and disrupts intermediate filaments dimerization leading to abnormal aggregation of proteins and cytoskeleton collapse [3, 10, 11]. GFAP identification and sequencing have increased the level of diagnosis accuracy and statistical analysis have evaluated the relationships between onset age and the GFAP genotype and its clinical outcomes [12]. Nearly all of the GFAP disease-causing mutations are heterozygous single base-pair alterations located in the coding region especially in central rod domain conserved α-helices. The remaining mutations are near the N-terminus precoil domain and C-terminal tail domain [3, 13]. In this study, we report a GFAP novel variant in a 2-year-old female affected with infantile form and conduct a comprehensive review on all of the reported GFAP mutations in patients with adult and juvenile forms as well.

**Methods**

**Case clinical features and demographic data**

A 2-year-old female patient referred to Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran, suffering from developmental delay and vomiting during one year after her birth. She was born through cesarean delivery and she was the only child of one healthy non-consanguineous parents (Fig. 1A). Her birth weight and head circumference were 2350 g and 33.9 cm, respectively. At age 24 months, she manifested some further symptoms including seizure and motor and speech delays. She could not also sit independently. The patient presented spasticity and increased deep tendon reflexes (DTRs). Further neurological examination also revealed ataxia and she had also gait disturbance. The clinical surveys of other available members of the pedigree were normal. After conducting clinical evaluations and family history recording and genetic counselling, whole-exome sequencing [14] was conducted for precise diagnosis. Identified candidate variant was confirmed and segregated in family members using PCR and direct Sanger sequencing. The study was performed in accordance with the Helsinki Declaration and has been approved by the Rajaei Cardiovascular, Medical, and Research Center ethics committee (IR.RHC.REC.1400.077).

**MRI**

Her first brain magnetic resonance imaging (MRI) at the age of 24 months indicated diffuse hyperintensity in periventricular and subcortical white matter of frontal and parietal lobes. Furthermore, basal ganglia indicated hyperintensity on apparent diffusion coefficient (ADC) maps. The brainstem and cerebellum had no abnormalities. Her MRI suggested leukodystrophy or hypoxic–ischemic encephalopathy. Her MRI reveals white matter involvement.

**Whole-exome sequencing**

Informed consent was obtained from the proband’s parents. DNA extraction was conducted according to salting out method. The quality and quantity of extracted DNA was checked by agarose gel electrophoresis and NanoDrop (Thermo Fisher Scientific, USA). DNA sample of the proband (III-1) (Fig. 1A) was subjected to WES and was conducted using at Macrogen (Seoul, South Korea) and raw data (fastq) was analyzed by Cardiogenetic Research Center, Rajaie Cardiovascular, Medical, and Research Center, Tehran, Iran.

The short reads alignment with human reference genome (UCSC build37/hg19) was performed by BWA (http://bio-bwa.sourceforge.net/) [15]. Any alterations including insertions/deletions (indels), single-nucleotide polymorphisms (SNPs) and polymerase chain reaction (PCR) duplicates removal were detected using Picard (http://picard.sourceforge.net/), SAMtools (http://www.htslib.org/) [16], and GATK (https://www.broadinstitute.org/gatk/) [17]. After annotation by annovar (http://annovar.openbioinformatics.org) [18], variants with minor allele frequency (MAF) < 0.05 were selected and filtered. In order to assess deleterious effects of variants, bioinformatics tools were applied including combined
Fig. 1 Genetic and protein changes of GFAP. A The pedigree of a family with Alexander disease. The black arrow indicates proband. Affected and unaffected individuals are represented by filled and clean symbols, respectively. B Sanger sequencing results show that a novel de novo variant in the GFAP was found in the proband (III-1) and normal sequence of her parents (II-4/II-5). C Conservation of p.Met73Val variant across various species has been shown. The variant site is highly conserved in various species. D, E Schematic view of GFAP and the position of mutation p.Met73Val.
Our genetic investigation revealed a novel de novo pathogenic variant, c.217A>G (p. Met73Val) in the recruited patient. Segregation analysis in the proband’s parents confirmed the identified variant of WES (Fig. 1B). The sequence alignments of proteins displayed the variant occurred within a highly conserved amino acid across various species, which provides its essential performance (Fig. 1C). Using schematic view of GFAP, the location of p.Met73Val was visualized. The identified variant is located on coil 1A of rod domain (Fig. 1D, E).

Bioinformatic analysis by different tools such as Mutation Taster, PROVEAN, PolyPhen-2, CADD, SIFT; and GERP categorized this variant as disease causing, neutral (Score: -1.540), possibly damaging (Score: 0.526), PHRED: 21.8, damaging (Score: 0.005), and Score: 3.73, respectively.

Our search strategy and data extraction led to collection of 86 articles that met our defined inclusion criteria. Totally 377 patients were recruited in these articles, among them 212 patients were affected with juvenile or adult-onset form carrier of an alteration in GFAP. 202 mutations were reported and among them 98 were unique (without duplication). c.262C>T 11/212 (5.18%), c.1246C>T 9/212 (4.24%), c.827G>T 8/212 (3.77%), c.232G>A 6/212 (2.83%) were more frequent comparing to other fulfilled mutations. Our search analysis revealed that bulbar signs 115/212 (54.24%), ataxia 74/212 (34.9%) and spasticity 59/212 (27.83%) were the dominant clinical symptoms among carrier of GFAP variants (Fig. 2).

According to our analysis, mutations located on coil2B (24.74%) and coil1A (23.71%) constituted the majority of reported mutations in juvenile and adult-onset forms (Table 2). Among these 98 unique fulfilled variants 54 and 35 variants were categorized as likely pathogenic and pathogenic, respectively (Table 2).

Discussion
Gain of function variants in GFAP are associated with different forms of AxD as a neurodegenerative disorder with autosomal dominant inheritance mode [3, 24]. GFAP is an important conserved intermediate filament protein with high expression level in astrocytes playing a significant role in central nervous system (CNS). Altered GFAP loses ability of extracellular K+ clearing and gliotic tissue hyperexcitability as the consequence [25]. This leads to astrocyte function impairment, demyelination changes and aggregation of Rosenthal fiber [26]. A comprehensive search on variants causing juvenile and adult was conducted and all the collected variants were analyzed by different in silico tools. Besides, our genetic analysis revealed a novel de novo variant in GFAP naming c.217A>G results in a methionine substitution to valine at codon 73 located in Coil 1A. GFAP-α (alpha) is the most abundant form of GFAP consists of head coil domain followed by the rod (filament) domain. Rod domain is also composed of four coils (1A, 1B, 2A, 2B). Reported variants near or within coil1A are Met73Lys, Met73Thr, and Met73Arg [13, 27–29]. Previous studies indicated that variants located within 1A, 1B and 2B domains may strongly cause severe form of AxD [13]. Met73Lys was first reported in a 7-month-old girl manifesting seizures and spasticity, but she did not indicate any bulbar signs or ataxia [27] and Met73Thr was reported in a 3-month-old girl. Her main clinical symptoms were macrocephaly, seizures,
### Table 1 Data extraction

| No. | Mutation | Protein change | Total recruited patients | Number of carriers | Age | DTR Ataxia | Hyper-tonia | Myoc. Enceph.-Scoliosis | Bulbar Nystagmus | Myoc. Palatal-spasticity | Status Epil. | Atrophy | Mental Retardation | Develop. Delay | Gait | Macro-cephaly | Shurred-Clums-Steadi. | Elec-mut. on one foot | Stand-On Ref |
|-----|----------|----------------|--------------------------|--------------------|-----|------------|-------------|----------------------|-----------------|-----------------------|-------------|---------|-------------------|----------------|------|--------------|-----------------------|---------------------------|---------------|
| 1   | c.214G > A | E72K          | 2                        | 2                  | 26  | 2          | 2           | 2                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
|     | c.1235C > T | T412I         | 3                        | 1                  | 33  | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 2   | c.731C > T | A244V         | 65                       | 1                  | 10  | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 3   | c.500G > A | R79H          | 1                        | 1                  | 6   | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 4   | c.988G > G  | p. Arg330Gly-p.Glu33 2Lys | 1                        | 4                  | 57  | 2          | 1           | 1                    | 1               | 2                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 5   | c.660G > G  | p.Q200E-p.R239C | 3                        | 1                  | 14  | 1          | 1           | 1                    | 2               | 2                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 6   | c.1157A > G | Gp.Asm38-p.Arg37 6Gl  | 1                        | 10                 | 2   | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 7   | c.235C > C  | p.R79C-p.R29C | 1                        | 10                 | 8   | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 8   | c.628G > A | A312K          | 1                        | 1                  | 10  | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 9   | c.500G > A | R79H          | 11                       | 1                  | 10  | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 10  | c.934G > T | E312K          | 1                        | 1                  | 67  | SM         |             |                     |                 |                       | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 11  | c.811G > C  | E263K          | 1                        | 40                 | 1   | 1          | 1           | 1                    | 1               | 1                    |            | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 12  | c.380G > A | D128N          | 1                        | 65                 | 1   | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| 13  | c.236G > C  | R79P          | 13                       | 5                  | 5   | 2          | 4           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
|     | c.1245C > T | R416W         | 13                       | 1                  | 13  | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
|     | c.1076T > C | L359P         | 19                       | 1                  | 19  | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
|     | c.290G > A  | R70Q          | 35                       | 1                  | 35  | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
|     | c.208C > T  | R70W          | 43                       | 1                  | 43  | 1          | 1           | 1                    | 1               | 1                    | 1           | 1       | 1                 | 1              | 1    | 1            | 1                     | 1                                   |               |
| No. | Mutation | Protein change | Total recruited of patients | Age | DTR Ataxia | Hyper-tonia | Myoc-Encep-Scoliosis | Bulbar Nysta-Palatal-spasticity | Status Epilepticus | Seizures | Atrophy | Mental Retardation | Develop. Mental Delay | Gait | Macro-cephaly | Slurred-Clums-Unsteadiness | Elective mutism | Standing on one foot | Other Ref |
|-----|----------|----------------|-----------------------------|-----|-------------|-------------|---------------------|--------------------------|-----------------|----------|---------|-------------------|---------------------|------|-----------------|--------------------------|---------------|---------------------|---------|
| 14  | c.53G>T  | p.Gly18Val     | 1                           | 46  | 1           |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     |         |
| 15  | c.382G>A | p.Aspl2Asn    | 1                           | 52  |             | RF          |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | RF [50] |
| 16  | c.219G>C  | p.M73I         | 1                           | 49  | 1           | 1           | 1                   |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | H [30]  |
| 17  | c.809G>C  | p.Arg270Pro    | 1                           | 36  | 1           | 1           | 1                   |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P [51]  |
| 18  | c.1245G>A | AM451I         | 3                           | 38  | 1           | 2           | 1                   |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | M (3) [52] |
| 19  | c.1076T>C | c.359P         | 11                          | 26  | 6           | 1           | 5                   |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | [53]   |
|     | c.1178G>T | p.S393I        |                             | 36  |             | RF          |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P        |
|     | c.1246C>T | p.R416W        |                             | 26  |             |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P        |
|     | c.209G>A  | p.R70Q         |                             | 39  |             |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P        |
|     | c.613G>A  | p.E205K        |                             | 30  |             |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P        |
|     | c.208C>T  | p.R70W         |                             | 43  |             |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P        |
|     | c.994G>A  | p.E332K        |                             | 61  |             |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P        |
|     | c.13G>A   | p.E205K        |                             | 58  |             |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P        |
|     | c.1193C>T | p.S398Y        |                             | 52  |             |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P        |
|     | c.380G>A  | p.D128N        |                             | 64  |             |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | P        |
| 20  | c.619C>G  | NA             | 3                           | 39  | 1           |             |                     |                          |                 |          |         |                   |                     |      |                 |                          |               |                     | M [54]   |
Table 1 (continued)

| No. | Mutation            | Protein change | Total recruited patients | Number of carriers | Age | DTR | Ataxia | Hypertonia | Myoclonus ataxiopathy | Spasticity | Encephalopathy | Bulbar-Nystagmus-myoclonus signs | Seizures | Atrophy | Mental retardation | Develop‑mental Delay | Macroglossia | Slurred speech | Unsteady gait | Clumsiness | Standing on one foot | Elective mutism | Other Ref |
|-----|---------------------|----------------|--------------------------|-------------------|-----|-----|--------|------------|-----------------------|-----------|---------------|-------------------------------|----------|---------|-------------------|------------------------|--------------|-----------------|--------------|-----------|---------------------|-----------------|----------|
| 21  | c.197G>A*          | p.Arg66        | 1 16 1 1 1 1           | 35                |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 22  | c.620A>T           | p.Glu20        | 1 52 1                | 55                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 23  | c.232G>C           | p.D78H         | 13                     | 13                 | 3   | 8   | 1      |            |                       |           | 1             |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 24  | c.219G>T           | p.M73I         | 1 48                   | 31                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 25  | c.1290G>A          | p.Arg43        | 3 42 2                | 56                 | 45  |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 26  | c.197G>A           | p.R66Q         | 1 1 54                 | 57                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 27  | c.799G>C           | p.A267P        | 1 25 8                | 1                   |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 28  | c.770AG            | p.Y257C        | 1 1 59                 | 59                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 29  | c.1079A>T          | p.D360V        | 1 1 9                  | 60                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 30  | c.1177A>C          | p.S393R        | 1 1 50 1              | 61                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 31  | 3 bp deletion      | -              | 1 1 8                  | 37                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 32  | c.302T>C           | L101P          | 1 1 26 1               | 62                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 33  | c.1290G>A          | p.Arg43        | 1 1 32 8              | 63                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 34  | c.1246C>T          | p.Arg4         | 1 1 1 1                | 64                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 35  | c.262C>T           | R80C           | 3 9 1 2 2 1           | 65                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
| 36  | c.382G>A           | p.Arg1         | 1 68 1                | 66                 |     |     |        |            |                       |           |               |                               |          |         |                   |                        |              |                 |              |           |                      |                  |          |
Table 1 (continued)

| No. | Mutation | Protein change | Total rec. mutated patients | Number of carriers | Age | DTR | Ataxia | Hypertonia | Myoclonus | Alopathy | Encephalopathy | Bulbar-Nystagmus | Palatal-Myoclonus | Status-Seizures | Epileptics | Atrophy | Mental Retardation | Develop-Gait Delay | Macrocephaly | Slurred-Speech | Clumsiness | Unsteadiness | Elective Mutism | Standing on one foot | Other | Ref |
|------|----------|----------------|----------------------------|-------------------|-----|-----|--------|-----------|-----------|----------|---------------|------------------|------------------|----------------|------------|---------|------------------|----------------|-------------|----------------|-----------|-------------|---------------|-------------------|-------|------|
| 37   | c.1246C>T | p.R416W        | 1                          | 1                  | 8   | 6   | 1      | 1         | 1         | 1        | 1             | 1                | 1                | 1              | 1         | 1       | 1                | 1              | 1           | 1              | 1         | 1           | 1             | 1                 | 1     | [67] |
| 38   | c.36T>C   | p.Leu12Pro     | 1                          | 1                  | 51  | 1   | 1      | 1         | 1         | 1        | 1             | 1                | 1                | 1              | 1         | 1       | 1                | 1              | 1           | 1              | 1         | 1           | 1             | 1                 |        | [68] |
| 39   | c.250G>A  | p.Arg79His     | 1                          | 1                  | 21  | 1   | 1      | 1         | 1         | 1        | 1             | 1                | 1                | 1              | 1         | 1       | 1                | 1              | 1           | 1              | 1         | 1           | 1             | 1                 |        | [69] |
| 40   | c.739T>C  | p.Ser24Pro     | 3                          | 3                  | 26  | 1   | 1      | 1         | 1         | 1        | 1             | 1                | 1                | 1              | 1         | 1       | 1                | 1              | 1           | 1              | 1         | 1           | 1             | 1                 |        | [70] |
| 41   | c.262C>T  | p.Arg88Cys     | 6                          | 6                  | 1   | 1   | 1      | 1         | 1         | 1        | 1             | 1                | 1                | 1              | 1         | 1       | 1                | 1              | 1           | 1              | 1         | 1           | 1             | 1                 |        | [71] |
| 42   | c.943G>T  | p.(E312*)      | 1                          | 1                  | 67  | 1   | 1      | 1         | 1         | 1        | 1             | 1                | 1                | 1              | 1         | 1       | 1                | 1              | 1           | 1              | 1         | 1           | 1             | 1                 |        | [72] |
| 43   | c.1087A>G | p.Arg365Val    | 1                          | 1                  | 3   | 1   | 1      | 1         | 1         | 1        | 1             | 1                | 1                | 1              | 1         | 1       | 1                | 1              | 1           | 1              | 1         | 1           | 1             | 1                 |        | [73] |
| 44   | c.827G>T  | R276L          | 3                          | 3                  | 33  | 1   | 1      | 1         | 1         | 1        | 1             | 1                | 1                | 1              | 1         | 1       | 1                | 1              | 1           | 1              | 1         | 1           | 1             | 1                 |        | [6]  |
| 45   | c.827G>T  | p.R276L        | 1                          | 1                  | 11  | 1   | 1      | 1         | 1         | 1        | 1             | 1                | 1                | 1              | 1         | 1       | 1                | 1              | 1           | 1              | 1         | 1           | 1             | 1                 |        | [74] |
Table 1 (continued)

| No. | Mutation | Protein change | Total recruited of carriers | Number of carriers | Age | DTR | Ataxia | Hyper-tonia | Myoclonus-halo-pathy | Encep-Scoliosis | Bulbar-Nystagmus | Ataxia | Hyper-tonia | Spasticity | Status epilepticus | Seizures | Mental retardation | Develop-Gait | Macrodcephaly | speech | Slurred | Unsteadiness | Clumsiness | Elective-mutism | Standing on one foot | Other Ref |
|-----|----------|----------------|----------------------------|--------------------|-----|-----|--------|-------------|-------------------|----------------|-------------------|---------|-------------|-----------|-------------------|---------|---------------------|-----------|-------------|--------|---------|-------------|------------|----------------|----------------------|-----------|
| 46  | c.827G>T | p.R276L        | 1                          | 1                  | 57  | 1   | 1      | 1           | 1                 | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 47  | c.1070G>T| L357P          | 1                          | 1                  | 7   |     |        | 1           |                   |                |                   |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 48  | c.262C>T | R88C           | 11                         | 1                  | 29  | 1   | 1      | 1           | 1                 | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 49  | c.617A>C | Glu206Ala      | 1                          | 1                  | 40  |     | 1      | 1           | 1                 | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 50  | c.724T>A | p.Y242N        | 1                          | 1                  | 37  | 2   | 2      | 1           | 1                 | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 51  | c.221T>C | M74T           | 1                          | 1                  | 50  | 1   |        |             |                   | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 52  | c.613G>A | p.E205K        | 30                         | 10                 | 10  | 2   | 4      | 5           | 4                 | 8              |                   |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 53  | c.211G>A | R66Q           | 30                         | 1                  | 40  | 1   |        |             |                   | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 54  | c.1179G>T| S393I          | 1                          | 1                  | 35  |     | 1      | 1           |                   |                |                   |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 55  | c.1100G>C | E362D         | 1                          | 1                  | 13  | 1   |        |             |                   | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 56  | c.355_3| p.Arg124_Leu125In | 1                        | 1                  | 13  | 1   |        |             |                   | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 57  | c.1148C>T | T383I         | 1                          | 1                  | 55  | 1   | 1      | 1           |                   | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 58  | c.1006T>C | L331P         | 1                          | 1                  | 7   |     |        |             |                   |                |                   |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| 59  | c.778A>C | p.Lys260Gln   | 1                          | 1                  | 25  | 1   |        |             |                   | 1              | 1                 |         |             |           |                   |         |                     |           |              |        |         |             |           |                 |                      |           |
| No. | Mutation | Protein change | Total recruited patients | Number of carriers | Age | DTR | Ataxia | Hyper-tonia | Myoclonus-halo-pathy | Encep-Scoliosis | Bulbar-Nystagmus | Myoclonus | Spasticity | Status-Seiz-Urinary | Mental retardation | Developmental Delay | Gait | Macrocephaly | Speech | Iness | Mutism | Slurred Clums-Unsteady | Stand-On | Other Ref |
|-----|----------|----------------|--------------------------|--------------------|-----|-----|--------|------------|-------------------|---------------|----------------|-----------|----------|-----------------|------------------|-------------------|------|------------|--------|-------|-------|---------------------|----------|----------|
| 60  | c.262C>T | p.R88C         | 22                       | 15                 |     | 6   | 9      | 4          |                   |               |             |           |          |                 |                  |                   |      |           |        |       |       |                     |         |          |
| 61  | c.1157A>G | p.N386S        | 1                        | 1                   | 72  | 1   | 1      | 1          |                   |               |             |           |          |                 |                  |                   |      |           |        |       |       |                     |         |          |
| 62  | c.803C>A  | A266D          | 19                       | 14                 | 8   | 13  | 14     |            |                   |               |             |           |          |                 |                  |                   |      |           |        |       |       |                     |         |          |
| 63  | c.262C>T | p.R88C         | 1                        | 1                   | 7   | 1   | 1      | 1          |                   |               |             |           |          |                 |                  |                   |      |           |        |       |       |                     |         |          |
| 64  | c.726_72 | p.E243dup      | 1                        | 1                   | 22  | 1   | 1      | 1          |                   |               |             |           |          |                 |                  |                   |      |           |        |       |       |                     |         |          |
| 65  | c.232G>A  | D78N           | 3                        | 3                   | 64  | 2   | 1      | 2          | 1                |               |             |           |          |                 |                  |                   |      |           |        |       |       |                     |         |          |
|     | c.232G>A  | D78N           | 55                       |                    |     |     |        |            |                   |               |             |           |          |                 |                  |                   |      |           |        |       |       |                     |         |          |
|     | c.232G>A  | D78N           | 32                       |                    |     |     |        |            |                   |               |             |           |          |                 |                  |                   |      |           |        |       |       |                     |         |          |
| No. | Mutation       | Protein change | Total Number | Age | DTR | Ataxia | Hyper-tonia | Myoclonus | Encephalopathy | Scissors | Bulbar-nystagmus | Spasticity | Status | Epilepsy | Mental Retardation | Developments | Gait | Macrocephy | Slurred | Clumsy | Unsteady | Elective Mutism | Other Refs |
|-----|----------------|----------------|--------------|-----|-----|--------|-------------|-----------|----------------|----------|----------------|------------|--------|----------|---------------------|--------------|------|-----------|---------|--------|---------|-----------------|-----------|
| 66  | c.61A>G        | p.His204Arg    | 66           | 1   | 1   | 55     | 1           | 1         | 1              |          | 1              | 1          |        |          |                      | H           |      |           |         |        |         |                 | [94]      |
| 67  | c.1246C>T      | p.R416W        | 67           | 1   | 1   | 7      | 1           | 1         |                |          |                |            |        |          |                      | C           |      |           |         |        |         |                 | [95]      |
| 68  | C1260T         | p.R416W        | 68           | 1   | 1   | 7      | 1           | 1         |                |          |                |            |        |          |                      |             |      |           |         |        |         |                 | [96]      |
| 69  | c.273G>C       | p.V87L         | 69           | 1   | 1   | 12     | 1           | 1         |                |          |                |            |        |          |                      | H           |      |           |         |        |         |                 | [97]      |
| 70  | c.273G>C       | p.V87G         | 70           | 1   | 2   | 7      | 1           | 1         |                |          |                |            |        |          |                      | (98)        |      |           |         |        |         |                 | [98]      |
| 71  | c.236G>A       | p.R79H         | 71           | 1   | 1   | 7      | 1           | 1         |                |          |                |            |        |          |                      | (2)         |      |           |         |        |         |                 |          |
| No. | Mutation | Protein change | Total Number | Age | Ataxia | Hyper‑tonia | Myoclonus | Encephalopathy | Bulbar signs | Nystagmus | Palatal‑spasticity | Status‑epilepticus | Seizures | Mental‑retardation | Developmental‑Delay | Mental‑retardation | Mental‑retardation | Clum‑Unsteadiness | Clum‑Unsteadiness | Clum‑Unsteadiness | Elective‑mutism | Other‑Ref |
|-----|----------|----------------|--------------|-----|---------|-------------|-----------|---------------|-------------|-----------|---------------------|-------------------|----------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 72  | c.619G>A  | Glu20         | 10           | 7   | 10      | 2           | 1         | 5             | 4           | 3         |                     |                   |          |                   |                   |                   |                   |                   |                   | [8]               |
|     | c.704T>C  | Ala24         |              |     |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   |                   | [96]              |
|     | c.715C>T  | Gly63         |              |     |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   |                   | [7]               |
| 73  | c.234C>G  | D78E          | 1            | 1   |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [99]              |
| 74  | c.1158C>A | N386K         | 1            | 1   |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [100]             |
| 75  | c.208C>T  | Arg70Trp      | 1            | 1   |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [101]             |
| 76  | c.380_38 | 5dupGC       | 7            | 2   | 1       | 1           | 1         |               | 2           | 1         |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [101]             |
|     | c.256_259delinsGGCT |             |              |     |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [101]             |
|     | c.256_259delinsGGCT |             |              |     |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [101]             |
|     | c.262C>T  | Gln34         |              |     |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [101]             |
|     | c.380_38 | 5dupGC       | 7            | 2   | 1       | 1           | 1         |               | 2           | 1         |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [101]             |
|     | c.256_259delinsGGCT |             |              |     |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [101]             |
|     | c.262C>T  | Arg94         |              |     |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [101]             |
| 77  | c.469G>A  | Asp157N       | 1            | 1   | 13      | 1           | 1         |               | 1           |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [102]             |
|     | c.1245G>A | Met4151       |              |     |         |             |           |               |             |           |                     |                   |          |                   |                   |                   |                   |                   |                   |                   |                   | [102]             |
| No. | Mutation        | Protein change | Total Number | Age | DTR | Ataxia | Hyper‑tonia | Myoclonus | Encephalopathy | Scoto‑sia | Bulbar signs | Nystagmus | Palatal‑myoclonus | Spasticity | Status epilepticus | Seizures | Atrophy | Mental retardation | Developmental Delay | Gait | Macro‑cephaly | Clumsiness | Dysarthria | Elective mutism | Standing on one foot | Other Ref |
|-----|----------------|----------------|--------------|-----|-----|--------|-------------|-----------|----------------|----------|--------------|-----------|-------------------|------------|-------------------|----------|---------|------------------|---------------------|-------|----------------|-----------|-----------|------------------|----------------------|---------|
| 78  | c.274T>G(c.274T>G87V) | 3   | 3   | 53  | 13  | 3     | 3           | 3         | 2              | 3        | 2             | 3         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
| 79  | c.1154 C>G  | p.Ser385Cys | 13  | 8   | 23  | 3     | 4           | 3         | 5              | 3        | 6             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
|     | c.259G>A  | NA          | 12  | 12  | 12  | 12     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
|     | c.715C>G  | p.Val87Ile  | 27  | 13  | 13  | 13     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
|     | c.701C>A  | p.Arg23Gly  | 44  | 39  | 39  | 39     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
|     | c.209 G>A  | p.Ala23Asp  | 33  | 33  | 33  | 33     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
|     | c.1118A>C  | p.Ser3Cys   | 7350  | 1    | 1    | 50    | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
| 80  | c.984C>G  | p.R329Glu   | 56  | 56  | 56  | 56     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
| 81  | c.994G>A  | p.E331K     | 1    | 1    | 1    | 1      | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
| 82  | c.236G>A  | p.R79His   | 36  | 36  | 36  | 36     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
| 83  | c.236G>A  | R79H       | 38  | 38  | 38  | 38     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
| 84  | c.232G>A  | p.D79N     | 1    | 1    | 1    | 1      | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
| 85  | c.221T>C  | p.M74T     | 1    | 1    | 1    | 1      | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
| 86  | c.1157A>G  | N386S     | 62  | 4    | 4    | 4      | 4           | 3         | 4              | 4        | 4             | 4         | 4               | 4          | 4                | 4         | 4               | 4               | 4               | 4     |
|     | c.628G>A  | E210K     | 58  | 58  | 58  | 58     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
|     | c.716G>A  | R258H     | 60  | 60  | 60  | 60     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
|     | c.208C>T  | R70W      | 64  | 64  | 64  | 64     | 1           | 1         | 1              | 1        | 1             | 1         | 1 teenager only | 4          | 1 teenager only   | 4         | 1 teenager only | 4               | 1 teenager only    | 4     |
| No. | Position on Chromosome (GRCh37) | HGVS DNA | HGVS protein | Exon/intron | SNP ID | Transcript | Coil | ClinVar | SIFT | Mutation | Taster | FATHMM | GERP | ACMG | CADD | PolyPhen-2 |
|-----|---------------------------------|----------|--------------|-------------|--------|------------|------|---------|------|----------|--------|--------|------|-------|------|----------|----------|
| 1   | 42987997                        | c.1157A>G | p.Asn386Ser  | E           | rs61726471 | ENST00000254308 | Tail | T       | DC   | N       | D      | 5.13   | LP   | 17.83 | B    |
| 2   | 42992647                        | c.208C>T  | p.Arg70Trp   | E           | rs60343255 | ENST00000254308 | Head | P       | D    | DC/P    | D      | 4.82   | P    | 24.1  | PD   |
| 3   | 42992549                        | c.306C>A  | p.Asn102Lys  | E           | -        | ENST00000254308 | Tail | T       | DC   | N       | T/D   | 4.69   | LP   | 21.8  | PD   |
| 4   | 42988006                        | c.1148C>T | p.Thr383Ile  | E           | rs267607517 | ENST00000254308 | Tail | P       | DC/P | D       | D      | 5.13   | LP   | 25.4  | PD   |
| 5   | 42992644                        | c.211G>A  | p.Ala71Thr   | E           | rs267607522 | ENST00000254308 | Head | DP      | D/P  | N       | D      | 4.82   | LP   | 23.1  | PD   |
| 6   | 42984686                        | c.*29C>T  | NA           | 3'UTR       | rs37008748 | ENST00000254308 | Tail | -       | -    | -       | -      | 5.07   | B    | -     | -    |
| 7   | 42986655                        | c.1076T>C | p.Leu399Pro  | E           | rs267607511 | ENST00000254308 | Coil2B | D      | D/P  | D       | D      | 4.25   | P    | 30    | PD   |
| 8   | 42986652                        | c.1079A>T | p.Asp360Val  | E           | rs6263501 | ENST00000254308 | Coil2B | D      | D/P  | D       | D      | 4.25   | LP   | 32    | PD   |
| 9   | 42986644                        | c.1087A>G | p.Lys363Val  | E           | -        | ENST00000254308 | Coil2B | D      | D      | DC/N   | D      | 4.25   | LP   | 27.3  | PD   |
| 10  | 42986641                        | c.1090G>A | p.Ala364Thr  | E           | rs58645997 | ENST00000254308 | Coil2B | P      | D    | DC      | D      | 4.25   | P    | 28.8  | PD   |
| 11  | 42986631                        | c.1100G>C | p.Arg367Thr  | E           | -        | ENST00000254308 | Coil2B | D    | D      | D       | 4.25   | P    | 28.8  | PD   |
| 12  | 42986613                        | c.1118A>C | p.Glu373Asp  | E           | rs79044589 | ENST00000254308 | Coil2B | P    | D    | DC      | D      | 4.25   | P    | 31    | PD   |
| 13  | 42986612                        | c.1119G>C | p.Glu373Asp  | E           | -        | ENST00000254308 | Coil2B | P    | D    | DC      | D      | 4.25   | P    | 25.6  | PD   |
| 14  | 42986605                        | c.1126C>T | p.Asp376Trp  | E           | rs267607512 | ENST00000254308 | Coil2B | P    | D    | DC      | D      | 4.25   | P    | 29.7  | PD   |
| 15  | 42986604                        | c.1127G>A | p.Asp376Gln  | E           | -        | ENST00000254308 | Coil2B | D    | D      | D       | 4.25   | P    | 36    | PD   |
| 16  | 42988000                        | c.1154C>G | p.Ser385Cys  | E           | rs79044590 | ENST00000254308 | Tail  | LP/P   | D    | DC      | D      | 5.13   | P    | 28.2  | PD   |
| 17  | 42987979                        | c.1157A>G | p.Asn386Ser  | E           | rs1276471 | ENST00000254308 | Tail  | T       | DC   | N       | D      | 5.13   | LP   | 17.83 | B    |
| 18  | 42987996                        | c.1158C>A | p.Asn386Lys  | E           | -        | ENST00000254308 | Tail  | T       | DC   | N       | D      | 5.13   | LP   | 24.9  | B    |
| 19  | 42985512                        | c.1177A>C | p.Ser393Arg  | E           | -        | ENST00000254308 | Tail  | T       | DC   | N       | -      | 5.23   | LP   | 22.6  | PD   |
| 20  | 42985511                        | c.1178G>T | p.Ser393Leu  | E           | rs62635764 | ENST00000254308 | Tail  | P    | T    | DC      | N       | 5.23   | LP   | 21.9  | B    |
### Table 2 (continued)

| No. | Position on Chromosome (GRCh37) | HGVS DNA | HGVS protein | Exon/Intron | SNP ID | Transcript | Coil | ClinVar | SIFT | Mutation Taster | PROVEAN | FATHMM | GERP | ACMG | CADD | PolyPhen-2 |
|-----|--------------------------------|----------|--------------|-------------|--------|------------|------|----------|------|----------------|---------|--------|------|------|------|-----------|
| 21  | 42985496 c.1193C > A          | p.Ser398Tyr | rs267607508  | ENST00000253408.5 | Tail   | P          | P    | DC       | N    | -          | 5.23    | LP      | 22.4 | PD   |
| 22  | 42985496 c.1193C > T          | p.Ser398Phe | rs267607508  | ENST00000253408.5 | Tail   | P          | D    | DC       | N    | -          | 5.23    | LP      | 22.7 | PD   |
| 23  | 42985454 c.1235C > T          | p.Asp412Ile | rs1597853099  | ENST00000253408.5 | Tail   | LP         | D    | DC       | D    | -          | 5.13    | LP      | 22.4 | PD   |
| 24  | 42985444 c.1245G > A          | p.Met415Ile | -            | ENST00000253408.5 | Tail   | -          | D    | P        | N    | -          | 5.13    | VUS/P  | 21.8 | B    |
| 25  | 42985439 c.1246C > T          | p.Arg416Trp | rs121909717  | ENST00000253408.5 | Tail   | P          | D    | DC       | D    | -          | 5.13    | LP      | 21.2 | PD   |
| 26  | 42984754 c.1260C > T          | p.Arg430His | rs779643685  | ENST00000253408.5 | Tail   | -          | -    | DC       | -    | -          | 4.80    | LB      | 18.95 | -    |
| 27  | 42984737 c.1277A > T          | p.Gln426Leu | rs775524073  | ENST00000253408.5 | Tail   | LP         | D    | DC       | D    | D          | 4.78    | LP      | 18.64 | PD   |
| 28  | 42984711 c.1290C > T          | p.Arg70Trp  | rs775524073  | ENST00000253408.5 | Tail   | LP         | D    | DC       | D    | P          | 4.78    | VUS/P  | 11.06 | -    |
| 29  | 42992668 c.187A > C           | p.Lys63Gln  | rs60095124   | ENST00000586793.1 | Head   | P          | D    | DC/P     | N    | D          | 4.82    | LP      | 23.5  | B    |
| 30  | 42992658 c.197G > A           | p.Arg66Gln  | rs797044569  | ENST00000586793.1 | Head   | Conflict   | D    | DC       | D    | D          | 5.89    | LP      | 29   | PD   |
| 31  | 42992647 c.201G > T           | p.Arg70Ile  | rs60343255   | ENST00000586793.1 | Head   | P          | D    | DC/P     | P    | D          | 4.82    | P       | 24.1  | PD   |
| 32  | 42992646 c.209G > A           | p.Arg70Gln  | rs60343255   | ENST00000586793.1 | Head   | P          | D    | DC/P     | P    | D          | 4.82    | P       | 22.2  | PD   |
| 33  | 42992641 c.214G > A           | p.Glu74Lys  | rs77607523   | ENST00000586793.1 | Head   | P          | D    | DC/P     | D    | D          | 4.82    | P       | 24   | B    |
| 34  | 42992636 c.219G > C           | p.Met78Ile  | rs60343255   | ENST00000586793.1 | Head   | P          | D    | DC/P     | D    | D          | 4.82    | P       | 23   | B    |
| 35  | 42992636 c.219G > T           | p.Met78Ile  | rs60343255   | ENST00000586793.1 | Head   | P          | D    | DC/P     | D    | D          | 4.82    | P       | 23   | B    |
| 36  | 42992634 c.221T > C           | p.Met78Thr  | rs60343255   | ENST00000586793.1 | Head   | P          | D    | DC/P     | D    | D          | 4.82    | P       | 22.3  | B    |
| 37  | 42992629 c.226C > T           | p.Leu79Phe  | rs75120761   | ENST00000586793.1 | Head   | P          | D    | DC/P     | D    | D          | 4.82    | P       | 26.7  | PD   |
| 38  | 42992624 c.231T > A           | p.Asn81Val  | rs75120761   | ENST00000586793.1 | Head   | P          | D    | DC/P     | D    | D          | 4.82    | P       | 23.3  | PD   |
| No. | Position on Chromosome 17 (GRCh37) | HGVS DNA | HGVS protein | Exon/ intron | SNP ID | Transcript | Coil | ClinVar | SIFT | Mutation Taster | PROVEAN | FATHMM | GERP | ACMG | CADD | PolyPhen-2 |
|-----|-----------------------------------|----------|--------------|-------------|--------|------------|------|----------|------|----------------|---------|---------|------|------|------|-----------|
| 41  | c.232G>A                          | p.Asp78Asn | E            | rs7970      | 44571  | ENST00000 586793.1 | Coil1A | P        | D    | DC            | D       | D       | 4.82 | P     | 26    | PD        |
| 42  | c.232G>C                          | p.Asp78His | E            | -           |        | ENST00000 591880.1 | Coil1A | -        | D    | DC            | D       | -       | 3.39 | VUS/P  | 26    | PD        |
| 43  | c.234C>G                          | p.Asp78Glu | E            | -           |        | ENST00000 586793.1 | Coil1A | -        | D    | DC            | D       | D       | 4.82 | P     | 26    | PD        |
| 44  | c.235C>T                          | p.Arg79Cys | E            | rs9793293   |        | ENST00000 586793.1 | Coil1A | P        | D    | DC            | D       | D       | 4.82 | P     | 24.9  | PD        |
| 45  | c.236G>A                          | p.Arg79His | E            | rs9285727   |        | ENST00000 586793.1 | Coil1A | P        | D    | DC            | D       | D       | 4.82 | P     | 24.6  | PD        |
| 46  | c.236G>C                          | p.Arg79Pro | E            | rs9285727   |        | ENST00000 586793.1 | Coil1A | P        | D    | DC            | D       | D       | 4.82 | P     | 26.8  | PD        |
| 47  | c.236G>T                          | p.Arg79Leu | E            | rs9285727   |        | ENST00000 586793.1 | Coil1A | P        | D    | DC            | D       | D       | 4.82 | P     | 26.7  | B         |
| 48  | c.250A>T                          | p.Ile84Phe | E            | -           |        | ENST00000 587997.1 | Coil1A | -        | D    | DC            | D       | D       | 5.07 | LP    | 24.3  | B         |
| 49  | c.256_259delinsGAGT               | p.Lys86_  | E            | rs267607501 |        | ENST00000 0586793.1 | Coil1A | P        | -    | -            | -       | -       | -    | -     | -     | -         |
| 50  | c.259G>A                          | p.Val87Ile | E            | rs267607518 |        | ENST00000 0586793.1 | Coil1A | P        | D    | DC            | N       | D       | 4.69 | P     | 24    | PD        |
| 51  | c.262C>T                          | p.Arg88Cys | E            | rs61622935  |        | ENST00000 0586793.1 | Coil1A | P        | D    | DC            | D       | D       | 4.69 | P     | 28.2  | PD        |
| 52  | c.262C>A                          | p.Arg88Ser | E            | rs61622935  |        | ENST00000 0586793.1 | Coil1A | P        | D    | DC            | D       | D       | 4.69 | P     | 31    | PD        |
| 53  | c.278A>C                          | p.Gln93Pro | E            | rs797044574 |        | ENST00000 586793.1 | Coil1A | P        | D    | DC            | D       | D/T     | 4.69 | LP    | 27.2  | PD        |
| 54  | c.302T>C                          | p.Leu101Pro | E            | rs267607516 |        | ENST00000 586793.1 | Coil1A | P        | D    | DC            | D       | D       | 4.69 | LP    | 24.3  | PD        |
| 55  | c.365_373dup                      | p.Arg124_ | E            | rs797044575 |        | ENST00000 586793.1 | Coil1B | -        | -    | -            | -       | -       | -    | -     | -     | -         |
| 56  | c.368T>C                          | p.Leu123Pro | E            | -          |        | ENST00000 586793.1 | Coil1B | -        | D    | DC            | D       | D       | 4.69 | LP    | 24.2  | PD        |
Table 2 (continued)

| No. | Position on Chromosome 17 (GRCh37) | HGVS DNA | HGVS protein | Exon/Intron | SNP ID | Transcript | Coil | ClinVar | SIFT | Mutation Taster | PROVEAN | FATHMM | GERP | ACMG | CADD | PolyPhen-2 |
|-----|-----------------------------------|----------|--------------|-------------|--------|------------|------|----------|------|-----------------|----------|--------|------|------|------|------------|
| 57  | 42992470                          | c.380_385dupCGGGCT | p.Leu127>Glu128dup | E           | --     | ENST00000586793.1 | CoilB | --       | --   | --              | --       | 45.7   | LP   | 24.2 | PD   |
| 58  | 42992473                          | c.382G>A  | p.Asp128Asn  | E           | rs267607509 | ENST00000586793.1 | CoilB | P        | D    | DC              | D        | D/T    | 4.57 | LP   | 24.2 | PD   |
| 59  | 42991449                          | c.469G>A  | p.Asp157Asn  | E           | rs59291670  | ENST00000586793.1 | CoilB | B        | D    | DC              | N        | D/T    | 5.55 | B    | 24.5 | B    |
| 60  | 42992802                          | c.53G>T   | p.Gly18Val   | E           | --     | ENST00000586793.1 | Head | T        | P    | N               | D        | 3.25   | VUS/P | 1.67 | B    |
| 61  | 42991103                          | c.611A>G  | p.His204Asp  | E           | --     | ENST00000586793.1 | CoilB | --       | D    | DC              | D        | 4.71   | LP   | 25   | PD   |
| 62  | 42991101                          | c.613G>A  | p.Glu205Lys  | E           | rs267607507 | ENST00000586793.1 | CoilB | P        | D    | DC              | D        | 4.71   | LP   | 25.1 | PD   |
| 63  | 42991097                          | c.617A>C  | p.Glu206Ala  | E           | --     | ENST00000586793.1 | CoilB | --       | D    | DC              | D        | 4.71   | P    | 33   | PD   |
| 64  | 42990798                          | c.619G>A  | p.Glu207Asp  | E           | rs267607500 | ENST00000586793.1 | CoilB | P        | D    | DC              | D        | 4.8    | P    | 34   | PD   |
| 65  | 42990798                          | c.619G>C  | p.Glu207Gln  | E           | rs267607500 | ENST00000586793.1 | CoilB | P        | D    | DC              | D        | 4.8    | P    | 33   | PD   |
| 66  | 42990797                          | c.620A>T  | p.Glu207Val  | E           | rs1555574517 | ENST00000586793.1 | CoilB | LP       | D    | DC              | D        | 4.8    | P    | 32   | PD   |
| 67  | 42990789                          | c.628G>A  | p.Glu210Lys  | E           | rs57661783  | ENST00000586793.1 | CoilB | P        | D    | DC              | D        | 4.92   | LP   | 31   | PD   |
| 68  | 42990725                          | c.692T>A  | p.Leu231His  | E           | rs797044577 | ENST00000586793.1 | Coil2A | P        | D    | DC              | D        | 4.92   | LP   | 24.9 | PD   |
| 69  | 42990713                          | c.704T>C  | p.Leu235Pro  | E           | rs60269890  | ENST00000586793.1 | Coil2A | P        | D    | DC              | D        | 4.92   | LP   | 24.9 | PD   |
| 70  | 42990702                          | c.715C>G  | p.Arg239Gly  | E           | rs58064122  | ENST00000586793.1 | Coil2A | VUS      | D    | DC              | D        | 4.92   | LP   | 25.3 | PD   |
| 71  | 42990702                          | c.715C>T  | p.Arg239Cys  | E           | rs58064122  | ENST00000586793.1 | Coil2A | P        | D    | DC              | D        | 4.92   | LP   | 25.3 | PD   |
| 72  | 42990701                          | c.716G>A  | p.Arg239His  | E           | rs59569590  | ENST00000586793.1 | Coil2A | P        | D    | DC              | D        | 4.92   | P    | 23.9 | PD   |
| 73  | 42990693                          | c.724T>A  | p.Tyr242Asn  | E           | --     | ENST00000586793.1 | Coil2A | --       | D    | DC              | D        | 4.92   | LP   | 25   | PD   |
| 74  | 42990686                          | c.731C>T  | p.Ala244Val  | E           | rs61497286 | ENST00000586793.1 | Coil2A | P        | D    | DC              | N        | 4.94   | LP   | 24.3 | PD   |
| 75  | 42990678                          | c.739T>C  | p.Ser247Pro  | E           | rs267607519 | ENST00000586793.1 | Coil2A | P        | D    | DC              | D/P       | 5.07   | LP   | 23.1 | PD   |
| 76  | 42990647                          | c.770A>G  | p.Tyr257Cys  | E           | rs26760750  | ENST00000586793.1 | Coil2B | P        | D    | DC              | D        | 5.07   | LP   | 25.5 | PD   |
| 77  | 42990639                          | c.778A>C  | p.Lys260Glu  | E           | --     | ENST00000586793.1 | Coil2B | -        | D    | DC              | D        | 5.07   | LP   | 28.9 | PD   |
| 78  | 42989147                          | c.799G>C  | p.Ala267Pro  | E           | rs79044581  | ENST00000586793.1 | Coil2B | P        | D    | DC              | D        | 4.42   | LP   | 27.1 | PD   |
| 79  | 42989143                          | c.803C>A  | p.Ala268Asp  | E           | rs79044582  | ENST00000586793.1 | Coil2B | P        | D    | DC              | D        | 4.42   | LP   | 25.7 | PD   |
| 80  | 42989137                          | c.809G>C  | p.Arg270Pro  | E           | --     | ENST00000586793.1 | Coil2B | --       | D    | DC              | D        | 4.42   | LP   | 25.2 | PD   |
| No.  | Position on Chromosome 17 (GRCh37) | HGVS DNA  | HGVS protein | Exon/intron | SNP ID   | Transcript | Coil | ClinVar | SIFT | Mutation Taster | PROVEAN | FATHMM | GERP | ACMG | CADD | PolyPhen-2 |
|------|-----------------------------------|-----------|--------------|-------------|----------|------------|------|---------|------|----------------|---------|--------|------|------|------|-----------|
| 81   | 42989119 c.827G>T                 | p.Arg276Leu E | rs121909719  | ENST00000586793.1 | Coil2B | P | D | DC | D | 4.42 | LP | 298 | PD |
| 82   | 42989078 c.868G>A                 | p.Gln290Glu E | rs797044583  | ENST00000586793.1 | Coil2B | P | D | DC | D | 4.38 | LP | 246 | PD |
| 83   | 42988797 c.934G>T                 | p.Glu312Ter E | rs763868966  | ENST00000586793.1 | Coil2B | VUS | - | DC | - | 4.65 | P | 22.8 | - |
| 84   | 42988743 c.988C>G                 | p.Arg330Gly E | rs267607513  | ENST00000586793.1 | Coil2B | P | D | DC | D | 4.51 | LP | 41  | PD |
| 85   | 42988737 c.994G>A                 | p.Glu332Lys E | rs267607514  | ENST00000586793.1 | Coil2B | P | D | DC | D | 4.51 | LP | 23.1 | PD |
| 86   | 42985511 c.1178G>T               | p.Ser393lel E | rs62635764   | ENST00000586793.1 | Coil2B | P | T | DC | N | 5.23 | LP | 24  | B  |
| 87   | 42992483 c.372-373insGAA        | p.Arg124_Leu125insGlu | - | ENST00000586793.1 | Coil1B | - | - | - | - | - | 4.63 | LP | 15.82 | - |
| 88   | 42990689 c.726_728dupAGG          | p.E243dup E | - | ENST00000586793.1 | Coil1B | - | - | - | - | - | 4.92 | LP | 16.67 | - |
| 89   | 42990716 c.701C>A                 | p.Ala234Asp E | rs1353739896  | ENST000005923201 | Coil2A | - | T | DC/P | D | D | 4.25 | LP | 19.72 | PD |
| 90   | 42990801 c.619-3C>G               | p.Ala234Asp I | rs112611995  | ENST00000586793.1 | - | P | - | - | - | - | VUS/P | - | - |
| 91   | 42992582 c.273A>C                 | p.Glu91Asp E | - | ENST00000586793.1 | Coil1A | - | D | DC | D | D | 4.69 | LP | 25.6 | PD |
| 92   | 42992581 c.274C>G                 | p.Gln92Glu E | - | ENST00000586793.1 | Coil1A | - | D | DC | N | D | 4.69 | LP | 24.9 | PD |
| 93   | 42992476 c.378_379dup            | p.Leu274ArgfsTer26 | - | ENST00000586793.1 | Coil1B | - | - | - | - | - | 4.58 | P | 17.26 | - |
| 94   | 42988612 c.1119G>C                | p.Glu373Asp E | - | ENST000004353602  | Coil2B | - | D | DC | D | D | 4.25 | P | 21.9 | PD |
| 95   | 42989044 c.902G>A                 | p.Gly301Asp E | - | ENST00000586793.1 | Coil2B | - | D | DC | D | D | 4.38 | LP | 25.3 | PD |
| 96   | 42990644 c.773G>C                 | p.Arg258Pro E | rs61726468  | ENST00000586793.1 | Coil2B | P | D | DC | D | 5.07 | LP | 26  | PD |
| 97   | 42989155 c.791T>C                 | p.Leu264Pro E | rs797044579  | ENST00000586793.1 | Coil2B | P | D | DC | D | 4.42 | LP | 25.3 | PD |
| 98   | 42992579 c.276G>T                | p.Gln290His E | - | ENST00000586793.1 | Coil1A | - | D | DC | D | D | 4.69 | LP | 24.5 | PD |

All the variants were analyzed based on the NM_002055, D damaging, T tolerated, DC disease causing, B benign, P polymorphism, LP likely pathogenic, P pathogenic, PD probably damaging, VUS variant of unknown significance, N neutral.
spasticity, bulbar signs, and ataxia [13]. Met73Arg is the third variant within this region and was reported in a patient with juvenile form. Her initial symptom was strabismus. In addition to the above-mentioned variants, Met73Ile and Met73Arg located in coil1A are also reported for patients affected with adult-onset form [30, 31]. Most of the reported mutations in GFAP gene are de novo and with 100% penetrance [3, 32]. A study conducted by Xiaoxuan Song et al. in 2021, two de novo mutations naming c.214G > A and c.1235C > T were reported in two unrelated individuals [33]. Both patients indicate regional neural activity increase. In
patients affected with AxD [36, 38]. This fact highlights the need for genetic analysis of AxD patients, especially in Iran, where published articles investigating the genetics of Iranian AxD patients are scarce [39]. There are only a few genetic markers identified so far in AxD patients [40]. These results indicate the importance of genetic studies in Iran and suggest the need for further investigation.

Accession Number
The accession number of the variant in ClinVar is as follows:
NM_002055.5(GFAP):c.217A > G(p.Met73Val):VCV001173085.1.

Acknowledgements
Special acknowledgments to the family that let us document their story to improve our realization of the condition.

Author contributions
KH wrote the initial manuscript text. NN and TM performed the wet lab evaluation. HP surveyed the patient clinically. SK contributed to the research design and analyzed WES data. All authors reviewed the manuscript.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This research was provided by the Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran, approved by RHC Ethics Committee (IR.RHC.REC.1400.077).

Informed consent
Informed consent has been obtained by the authors.

Competing interests
The authors declare that they have no conflict of financial interest.

Author details
1Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.
2Cardiovascular Imaging Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.

Received: 21 May 2022 Accepted: 23 August 2022
Published online: 10 September 2022

References
1. Barkovich AJ, Messing A. Alexander disease: not just a leukodystrophy anymore. In: AAN Enterprises; 2006.
2. Yoshida T, Sasaki M, Yoshida M, Namekawa M, Okamoto Y, Tsujiino S, et al. Nationwide survey of Alexander disease in Japan and proposed new guidelines for diagnosis. J Neurol. 2011;258(11):1998–2008.
3. Brenner M, Johnson AB, Boespflug-Tanguy O, Rodriguez D, Goldman JE, Messing A. Mutations in GFAP encoding glial fibrillary acidic protein, are associated with Alexander disease. Nat Genet. 2001;27(1):117–20.
4. Springer S, Erlewein R, Naegeli I, Becker I, Aufer D, Grodd W, et al. Alexander disease-clasification revisited and isolation of a neonatal form. Neuropediatrics. 2000;31(02):86–92.
5. Johnson AB. Alexander disease: a review and the gene. Int J Dev Neurosci. 2002;20(3–5):391–4.
6. Namekawa M, Taktyama Y, Aoki Y, Takayayshiki N, Sakoe K, Shimazaki H, et al. Identification of GFAP gene mutation in hereditary adult-onset Alexander’s disease. Ann Neurol. 2002;52(6):779–85. https://doi.org/10.1002/ana.10375.
7. van der Knaap MS, Ramesh V, Schiffmann R, Blaser S, Kylleman M, Gholkar A, et al. Alexander disease: ventricular garlands and abnormalities of the medulla and spinal cord. Neurology. 2006;66(4):494–8. https://doi.org/10.1212/01.wnl.0000198770.80743.37.
8. van der Knaap MS, Salomons GS, Li R, Franceszi E, Gutiérrez-Solano LG, Smit LM, et al. Unusual variants of Alexander disease. Ann Neurol. 2005;57(3):372–8. https://doi.org/10.1002/ana.20381.
9. Paprocka J, Rzepeka-Migut B, Rzepeka N, Jezela-Stanek A, Morava E. Infantile Alexander disease with late onset infantile spasms and hypsarrhythmia. Balkan J Med Genet. 2019;22(2):77.
10. Eng LF, Ghimikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000). Neurochem Res. 2000;25(9):1439–51.
11. Nielsen AL, Jørgensen P, Jørgensen AL. Mutations associated with a childhood leukodystrophy, Alexander disease, cause deficiency in dimerization of the cytoskeletal protein GFAP. J Neurogenet. 2002;16(3):175–9. https://doi.org/10.1080/01677060215305.
12. Prust M, Wang J, Morizono H, Messing A, Brenner M, Gordon E, et al. GFAP mutations, age at onset, and clinical subtypes in Alexander disease. Neurology. 2011;77(15):1287–94.
13. Li R, Johnson AB, Salomons G, Goldman JE, Naidu S, Quinnan R, et al. Glial fibrillary acidic protein mutations in infantile, juvenile, and adult forms of Alexander disease. Ann Neurol. 2005;57(3):310–26. https://doi.org/10.1002/ana.20406.
14. Rowczynio DM, Noor I, Gillmore JD, Lachmann HJ, Whelan C, Hawkins PN, et al. Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. Hum Mutat. 2014;35(9):E2403–12.

15. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(16):1754–60.

16. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. Bioinformatics. 2009;25(16):2078–9.

17. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a mapreduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20(9):1297–303.

18. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of variants from high-throughput sequencing data. Nucl Acids Res. 2010;38(16):e164–e.

19. Kircher M, Witten DM, Jain P, O’roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014;46(3):310–5.

20. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4(7):1073–81.

21. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014;11(4):366–9.

22. Choo Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics. 2015;31(16):2745–7.

23. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PhyloPhein-2. Curr Protoc Hum Genet. 2013;76:1.7.20.1–7.

24. van der Knaap MS, Naidu S, Breiter SN, Blaser S, Stroink H, Springer S, et al. Independent mechanisms of potassium clearance in Alexander disease. J Pediatr. 2013;163(5):481–4.

25. Walz W, Wuttke WA. Decreased dopaminergic uptake in dopamine transporter scintigraphy. case of Alexander disease presented with dystonia of lower limb and increased towel stiffness. Rinsho shinkeigaku Clin Neurol. 2020;60(10):712–5. https://doi.org/10.1589/0883073815435838.

26. Bickenzi K, Haghi Panah M, Tavasoli AR, Ashrafi MR, Mahdieh PN, et al. Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. Hum Mutat. 2014;35(9):E2403–12.

27. Posey JE, Harel T, Liu P, Rosenfeld JA, James RA, Coban Akdemir ZH, et al. A novel mutation in glial fibrillary acidic protein gene in a patient with Alexander disease. Neurosci Lett. 2001;312(2):71–4. https://doi.org/10.1016/S0304-3940(00)02139-5.

28. Asahina N, Okamoto T, Sudo A, Kanazawa N, Tsuchio S, Saitoh S. An infantile-juvenile form of Alexander disease caused by a R79H mutation in GFAP gene. Brain Dev. 2006;28(2):131–3. https://doi.org/10.1016/j.braindev.2005.05.004.

29. Balbi P, Seri M, Ceccherini I, Uggetti C, Casale R, Fundaro C, et al. Adult-onset Alexander disease: report of a patient. Neurol. 2008;25(1):24–30. https://doi.org/10.1111/j.1469-8686.2007.05680.x.

30. Barreau P, Prust MJ, Crane J, Loewenstein J, Kadom N, Vanderave K. Focal central white matter lesions in Alexander disease. J Child Neurol. 2011;26(1):1422–4. https://doi.org/10.1177/1091256711405381.

31. Bianchini L, Aquino D, No Di Bella D, Sarto E, Moscatelli M, Pareyson D, et al. Severe worsening of adult-onset Alexander disease after minor head trauma: report of two patients and review of the literature. J Clin Neurosci. 2020;75:221–3. https://doi.org/10.1016/j.jocn.2020.03.033.

32. Bianchini L, Rossi A, Ceccherini I, Pezzella M, Prato G, Striano P, et al. Magnetic resonance imaging “tigroid pattern” in Alexander disease. Neuropediatrics. 2013;44(3):174–6. https://doi.org/10.1055/s-0032-1329910.

33. Bonthius DJ, Karacay B. Alexander disease: a novel mutation in GFAP leading to Epilepsia Partialis Continua. J Child Neurol. 2016;31(7):869–72. https://doi.org/10.1177/0883073815624762.

34. Brenner M, Blessing A. A new mutation in GFAP widens the spectrum of Alexander disease. Eur J Hum Genet. 2015;23(1):1–2. https://doi.org/10.1038/ejhg.2014.99.

35. Brockmann K, Meins M, Taubert A, Trappe R, Grond M, Hanefeld F. A novel GFAP mutation and disseminated white matter lesions: adult Alexander disease? Eur Neurol. 2003;50(2):100–5. https://doi.org/10.1159/000072507.

36. Cabrera-Galván JJ, Martínez-Martin MS, Déniz-García A, Araujo-Ruano E, Travesio-Aja MD. Adult-onset Alexander disease with a heterozygous D128N GFAP mutation: a pathological study. HistoPathology. 2019;34(9):1073–188. https://doi.org/10.1111/his.13180.

37. Casasnovas C, Verdua E, Velez V, Schluter A, Pons-Escoda A, Homedes C, et al. A novel mutation in the GFAP gene expands the phenotype of Alexander disease. J Med Genet. 2019;56(12):846–9. https://doi.org/10.1136/jmedgenet-2018-105959.

38. Chang KE, Pratt D, Mishra BB, Edwards N, Hanefeld F. Type II (adult-onset) Alexander disease in a paraplegic male with a rare D128N mutation in the GFAP gene. Clin Neuropathol. 2015;34(5):298–302. https://doi.org/10.1111/cnp.2015.34.issue-5.

39. de Paiva AR, Freua F, Lucato LT, Parmera J, Dória D, Nóbrega PR, et al. A novel GFAP mutation in a type II (late-onset) Alexander disease patient. J Neurol. 2016;263(4):821–2. https://doi.org/10.1007/s00415-016-8065-8.

40. Elmali AD, Çetinçilik U, İşlak C, Uzun Adatpe N, Karaali Savrun F, Yalçınkaya C. Familial adult-onset Alexander disease: clinical and neuroradiological findings of three cases. Neuro Psikiyatr Arş. 2016;33(2):169–72. https://doi.org/10.5152/npa.2015.10193.

41. Farina L, Pareyson D, Minati L, Ceccherini I, Chiapparini L, Romano S, et al. Can MR imaging diagnose adult-onset Alexander disease? AJNR Am J Neuroradiol. 2008;29(6):1190–6. https://doi.org/10.3174/ajnr.a1080.

42. Flint D, L R, Webster LS, Rabbani B. GFAP variants leading to infantile Alexander disease: report of four unrelated patients and review of the literature. Ped Radiol. 2012;33(7):1141–8. https://doi.org/10.1007/s00499-012-22094.

Phenotype and genotype analysis of 135 cases and report of a de novo variant. Clin Neurol Neurosurg. 2021;207:10675–4. https://doi.org/10.1016/j.clineuro.2021.106754.
55. Gass JM, Cheema A, Jackson J, Blackburn PR, Van Gerpen J, Atwal PS, et al. European Journal of Medical Research (2022) 27:174

57. Hida A, Ishiura H, Arai N, Fukuoka H, Hasuo K, Goto J, et al. Adult-onset Alexander disease with an R66Q mutation in GFAP presented with severe vocal cord paralysis during sleep. J Neurol. 2012;259(12):2346–5. https://doi.org/10.1007/s00415-012-5640-4.

58. Hirata R, Kattunen V, Kattunen A, Herva R, Uusimaa J, Remes AM. Alexander disease with ocular predominance and a novel c.799G>C mutation in the GFAP gene. Acta Neuropathol. 2007;114(5):543–5. https://doi.org/10.1007/s00401-007-0292-8.

59. Howard KL, Hall DA, Moon M, Agarwal P, Newman E, Brenner M. Adult-onset Alexander disease with progressive ataxia and palatal tremor. Mov Disord. 2008;23(1):118–22. https://doi.org/10.1002/mds.21774.

60. Ishigaki K, Ito Y, Sawayashi Y, Kodaira K, Funatsuka M, Hattori N, et al. TRH therapy in a patient with juvenile Alexander disease. Brain Dev. 2006;28(10):663–7. https://doi.org/10.1016/j.braindev.2006.05.001.

61. Iwasaki Y, Saito Y, Mori K, Ito M, Mimuro M, Aiba I, et al. An autopsied case of adult-onset bulbospinal form Alexander disease with a novel S393R mutation in the GFAP gene. Clin Neuropathol. 2015;34(4):207–14. https://doi.org/10.5414/np00808.

62. Kaneko H, Hirose M, Katada S, Takahashi T, Naruse S, Tsuchiya M, et al. Novel GFAP mutation in patient with adult-onset Alexander disease presenting with spastic ataxia. Mov Disord. 2009;24(9):1393–5. https://doi.org/10.1002/mds.22556.

63. Karp N, Lee D, Shickh S, Jenkins ME. c.1289G>A (p.Arg430His) variant on the fibrillary acidic protein gene mutation. Neurosci Lett. 2003;350(3):169–72. https://doi.org/10.1016/s0304-3940(03)00900-5.

64. Kinoshita T, Imaizumi T, Miura Y, Fujimoto H, Ayabe M, Shoji H, et al. A case of adult-onset Alexander disease with dropped head syndrome. Rinsho shinkeigaku. 2011. 1181.

65. Kyllerman M, Rosengren L, Wiklund LM, Holmberg E. Increased levels of GFAP in the cerebrospinal fluid in three subtypes of genetically confirmed Alexander disease. Neurodiagnostic Medicine. 2004;35(3):169–72. https://doi.org/10.1016/j.jnd.2004.07.006.

66. Lee SH, Nam TS, Kim KH, Kim JH, Yoon W, Heo SH, et al. Aggregation-prone GFAP mutation in Alexander disease validated using a zebrafish model. BMC Neurol. 2017;17(1):175. https://doi.org/10.1186/s12883-017-0938-7.

67. Liu Y, Zhou H, Wang H, Gong X, Zhou A, Zhao L, et al. Athyrial MRI features in familial adult-onset Alexander disease: case report. BMC Neurol. 2016;16(1):211. https://doi.org/10.1186/s12883-016-0734-9.

68. Maeda K, Iwai K, Kobayashi Y, Tsuji H, Yoshida T, Kobayashi Y. A case of adult-onset Alexander disease with a novel mutation in the GFAP gene. J Neurol. 2007;254(9):1278–80. https://doi.org/10.1007/s00415-006-0361-2.

69. Namekawa M, Takiyama Y, Honda J, Shimazaki H, Sakoe K, Nakano I. A novel adult-onset Alexander disease with typical “tadpole” brainstem atrophy and unusual bilateral basal ganglia involvement: a case report and review of the literature. BMC Neurol. 2010;10:21. https://doi.org/10.1186/1471-2377-10-21.

70. Niinikoski H, Haataja L, Brandner A, Valanne L, Blaser S. Alexander disease as a cause of nocturnal vomiting in a 7-year-old girl. Pediatr Radiol. 2009;39(8):785–9. https://doi.org/10.1007/s00247-009-0987-8.

71. Nobuhara Y, Nakahara K, Higuchi I, Yoshida T, Fushiki S, Osame M, et al. Juvenile form of Alexander disease with GFAP mutation and mitochondrial abnormality. Neurology. 2004;63(7):1302–4. https://doi.org/10.1212/01.wnl.0000140695.90497.e2.

72. Ogawa T, Ogaki K, Ishiguro M, Ando M, Yoshida T, Noda K, et al. Novel GFAP p. Glu206Ala mutation in Alexander disease with decreased dopamine transporter uptake. Mov Disord Clin Pract. 2020;7(6):720–2. https://doi.org/10.1002/mdc3.12998.

73. Ogura H, Maki F, Sasaki N, Yoshida T, Hasegawa Y. Familial adult-onset Alexander disease with a Novel GFAP mutation. Mov Disord Clin Pract. 2016(3):300–2. https://doi.org/10.1002/mdc3.12296.

74. Ohnari K, Yamano M, Uozumi T, Hashimoto T, Tsuji S, Nakagawa M. An adult form of Alexander disease: a novel mutation in granulofilibrillary acidic protein. J Neurol. 2007;254(10):1390–4. https://doi.org/10.1007/s00415-007-0557-0.

75. Pareyson D, Fancellu R, Mariotti C, Romano S, Salmaggi A, Carella F, et al. Adult-onset Alexander disease: a series of eleven unrelated cases with review of the literature. Brain. 2008;131(Pt 9):2321–31. https://doi.org/10.1093/brain/awn178.

76. Salmaggi A, Bottu A, Lamperti E, Grisoli M, Fischetto R, Ceccerini I, et al. A novel mutation in the GFAP gene in a familial adult onset Alexander disease. J Neurol. 2007;254(9):1278–80. https://doi.org/10.1007/s00415-006-0361-2.

77. Sawaishi Y, Yano T, Takada I, Takada G. Juvenile Alexander disease with a novel mutation in glial fibrillary acidic protein gene. Neurology. 2002;58(10):1541–3. https://doi.org/10.1212/01.wnl.0000105417.14517.10.

78. Schmidt H, Kretzschmar B, Lingor P, Pauli S, Schramm P, Otto M, et al. Acute onset of adult Alexander disease. J Neurol Sci. 2013;311(1–2):152–4. https://doi.org/10.1016/j.jns.2013.05.006.

79. Schmidt S, Wattjes MP, Gerding WM, van der Knaap M. Late onset Alexander's disease presenting as cerebellar ataxia associated with a novel mutation in the GFAP gene. J Neurol. 2007;255(5):938–40. https://doi.org/10.1007/s00415-010-5849-0.

80. Shihihara T, Sawaishi Y, Adachi M, Kato M, Hayasaki K. Asymptomatic hereditary Alexander's disease caused by a novel mutation in GFAP. J Neurol Sci. 2004;225(1–2):125–7. https://doi.org/10.1016/j.jns.2004.07.008.

81. Zaver DB, Douthit NT. A novel mutation in the adult-onset Alexander's disease GFAP gene. Case Rep Med. 2019;2019:2968538. https://doi.org/10.1155/2019/2968538.

82. Zang L, Wang J, Jiang Y, Gu Q, Gao Z, Yang Y, et al. Follow-up study of 22 Chinese children with Alexander disease and analysis of parental origin of de novo GFAP mutations. J Hum Genet. 2013;58(4):183–8. https://doi.org/10.1038/jhg.2012.152.

83. Sugiyama A, Sawai S, Ito S, Mukai H, Beppu M, Yoshida T, et al. Incidental diagnosis of an asymptomatic adult-onset Alexander disease by brain magnetic resonance imaging for preoperative evaluation. J Neurol Sci. 2015;354:131–2.

84. Yoshida T, Yasuda R, Mizuta I, Nakagawa M, Mizuno T. Quantitative evaluation of brain stem atrophy using magnetic resonance imaging in adult patients with Alexander disease. Eur Neurol. 2017;77(5–6):296–302. https://doi.org/10.1159/000475661.
91. Ye W, Qiang G, Jingmin W, Yanling Y, Xiru W, Yuwu J. Clinical and genetic study in Chinese patients with Alexander disease. J Child Neurol. 2008;23(2):173–7. https://doi.org/10.1177/0883073807308691.
92. Yasuda R, Yoshida T, Mizuta I, Nakagawa M, Mizuno T. A novel three-base duplication, E243dup, of GFAP identified in a patient with Alexander disease. Hum Genome Var. 2017;4:17028. https://doi.org/10.1038/hgv.2017.28.
93. Wada Y, Yanagihara C, Nishimura Y, Namekawa M. Familial adult-onset Alexander disease with a novel mutation (D78N) in the glial fibrillary acidic protein gene with unusual bilateral basal ganglia involvement. J Neurol Sci. 2013;331(1–2):161–4. https://doi.org/10.1016/j.jns.2013.05.019.
94. Vázquez-Justes D, Peñalva-García J, López R, Mitjana R, Begue R, González-Mingot C. Parkinsonism phenotype in a family with adult onset Alexander disease and a novel mutation of GFAP. Clin Neurol Neurosurg. 2020;195:105893. https://doi.org/10.1016/j.clineuro.2020.105893.
95. Tulyeu J, Tamaura M, Jimbo E, Shimbho H, Takano K, Iai M, et al. Aggregate formation analysis of GFAP(R416W) found in one case of Alexander disease. Brain Dev. 2019;41(2):195–200. https://doi.org/10.1016/j.braindev.2018.08.009.
96. Thyagarajan D, Chataway T, Li R, Gai WP, Brenner M. Dominantly-inherited adult-onset leukodystrophy with palatal tremor caused by a mutation in the glial fibrillary acidic protein gene. Mov Disorder. 2004;19(10):1244–8. https://doi.org/10.1002/mds.20161.
97. Suzuki H, Yoshida T, Kitada M, Ichihashi J, Sasayama H, Nishikawa Y, et al. Late-onset Alexander disease with a V87L mutation in glial fibrillary acidic protein (GFAP) and calcifying lesions in the sub-cortex and cortex. J Neurol. 2012;259(3):457–61. https://doi.org/10.1007/s00415-011-6201-z.
98. Yoshida T, Sasayama H, Mizuta I, Okamoto Y, Yoshida M, Riku Y, et al. Glial fibrillary acidic protein mutations in adult-onset Alexander disease: clinical features observed in 12 Japanese patients. Acta Neurol Scand. 2011;124(2):104–8. https://doi.org/10.1111/j.1600-0404.2010.01427.x.
99. Stumpf E, Masson H, Duquette A, Berthelet F, McNabb J, Lortie A, et al. Adult Alexander disease with autosomal dominant transmission: a distinct entity caused by mutation in the glial fibrillary acid protein gene. Arch Neurol. 2003;60(9):1307–12. https://doi.org/10.1001/archneur.60.9.1307.
100. Stitt DW, Gavrilova R, Watson R, Hassan A. An unusual presentation of late-onset Alexander's disease with slow orthostatic tremor and a novel GFAP variant. Neurocase. 2018;24(5–6):619–25. https://doi.org/10.1080/17432940.2018.1535326.
101. Sreedharan J, Shaw CE, Jarosz J, Samuel M. Alexander disease with slow orthostatic tremor and a novel GFAP variant. Neurocase. 2018;24(5–6):619–25. https://doi.org/10.1080/17432940.2018.1535326.
102. Rezende SADS, Fernandes M, Munhoz RP, Raskin S, Schelp AO, Knaap KM, et al. Familial adult-onset Alexander disease featuring severe atrophy of the medulla oblongata and upper cervical cord on magnetic resonance imaging. Case Rep Neurol. 2012;4(3):202–6. https://doi.org/10.1159/000345303.
103. Okamoto Y, Mitsuyama H, Jonosono M, Hirata K, Arimura K, Osame M, et al. Familial adult-onset Alexander disease suspected juvenile-onset and exacerbating after long stationary state. Rinsho shinkeigaku Clin Neurol. 2013;53(6):474–7. https://doi.org/10.5692/clinicalneurol.53.474.
104. Yonezu T, Ito S, Kanai K, Masuda S, Shibuya K, Kuvabara S. A case of adult-onset Alexander disease featuring severe atrophy of the medulla oblongata and upper cervical cord on magnetic resonance imaging. Case Rep Neurol. 2012;4(3):202–6. https://doi.org/10.1159/000345303.
105. Yoshida T, Mizuta I, Saito K, Kimura Y, Park K, Ito Y, et al. Characteristic abnormal signals in medulla oblongata-“eye spot” sign: four cases of elderly-onset Alexander disease. Neurol Clin Pract. 2015;5(3):259–62. https://doi.org/10.1212/cpne.0000000000000124.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions