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# Description of Phenotypic Heterogeneity in a *GJC2*-Related Family and Literature Review

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**Keywords**
*GJC2* gene · Hereditary spastic paraplegia · Hypomyelinating leukodystrophy 2 · Pelizaeus-Merzbacher-like disease-1 · Phenotypic heterogeneity · SPG44

**Abstract**
***Introduction:*** Homozygous and compound heterozygous variants in *GJC2*, the gene encoding connexin-47 protein, cause Pelizaeus-Merzbacher-like disease type 1 or hypomyelinating leukodystrophy 2 (HLD2), a severe infantile-onset hypomyelinating leukodystrophy, and rarely some milder phenotypes like hereditary spastic paraplegia (HSP) type 44 (SPG44) and subclinical leukodystrophy. Herein, we report an Iranian *GJC2*-related family with intrafamilial phenotypic heterogeneity and review the literatures. ***Methods:*** Whole-exome sequencing was performed for an Iranian proband, who was initially diagnosed as HSP case. Data were analyzed and the candidate variant was confirmed by PCR and Sanger sequencing subsequently checked in family members to co-segregation analysis. A careful clinical and paraclinical evaluation of all affected individuals of the family was done and compared with previous reported *GJC2*-related families. ***Results:*** A novel homozygous variant, c.G14T:p.Ser5Ile, in the *GJC2* gene was identified. The variant was co-segregated with the disease status in the family members. Clinical evaluation of all patients showed two distinct *GJC2*-related phenotypes in this family; the proband presented a complicated form of HSP, whereas both his affected sisters presented a HLD2 phenotype. ***Discussion:*** Up to now, correlation between HSP and *GJC2* variants has been reported once. Here, the second case of SPG44 was identified that emphasizes on *GJC2* as a HSP-causing gene. So, the screening of *GJC2* in patients with HSP or HSP-like phenotypes especially with hypomyelination in their brain MRI is recommended. Also, for the first time, intrafamilial phenotypic heterogeneity for "two distinct *GJC2*-related phenotypes: HLD2 and HSP" was reported. Such intrafamilial phenotypic heterogeneity for *GJC2* can emphasize on the shared pathophysiology of these disorders.


## Introduction

The *GJC2* gene, gap junction protein gamma 2/*GJA12*: gap junction protein alpha 12: OMIM: 608803, located on chromosome 1q42.13 [Lo Giudice et al., 2014; Owczarek-Lipska et al., 2019], is expressed only in oligodendrocytes within the nervous system [Abrams, 2019]. The gene con-

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tains a single coding exon and encodes the connexin-47 (Cx47) protein with 439 amino acids [Owczarek-Lipska et al., 2019]. Cx47 belongs to a highly conserved membrane protein family of connexins and constitutes nine domains: four transmembrane, two extracellular, and three cytoplasmic domains including N- and C-termini and cytoplasmic loop [Hobson and Garbern, 2012; Javadikooshesh et al., 2021]. The protein forms gap junctions – a type of intercellular channel, resulting in direct cell-to-cell diffusion of ions and small molecules [Lo Giudice et al., 2014].

Mutations in the *GJC2* gene which are related to hypomyelinating leukodystrophy 2 (HLD2), OMIM #608804, or Pelizaeus-Merzbacher-like disease-1 were reported by Uhlenberg et al. [2004] for the first time. HLD2 is mainly characterized by motor and speech delay and nystagmus which gradually evolves to progressive spasticity, choreoathetiod movements, and ataxia [Wang et al., 2010]. Nowadays, more than 80 variants have been identified in the *GJC2* gene (according to HGMD professional 2021.4; https://www.hgmd.cf.ac.uk/ac/index.php); most of them are associated with HLD2 [Uhlenberg et al., 2004; Wang et al., 2010; Owczarek-Lipska et al., 2019], while a few are related to other phenotypes such as spastic paraplegia 44 (SPG44), autosomal recessive, OMIM #613206 [Orthmann-Murphy et al., 2009], or hereditary lymphedema [Ferrell et al., 2010; Finegold et al., 2012] (online suppl. Table S1 www.karger.com/doi/10.1159/000529678). Almost all variants in *GJC2*, which are associated with neurological disorders, present a recessive pattern of inheritance, except three mutations with a probably dominant inheritance that have been detected in four HLD2 probands; the second mutations have not been identified in these cases [Diekmann et al., 2010]. Interestingly, there are also some reports that HLD2 is caused by uniparental disomy [Wang et al., 2010; Shimojima et al., 2013]. Investigation of genotype-phenotype correlations shows that the *GJC2* mutations which result in complete loss of the Cx47 protein function as well as mutations which affect the *GJC2* gene promoter area mainly resulted in the severe phenotypes of HLD2, while the *GJC2* mutations which are leading to a partial loss of function and/or mild protein alterations seem to cause less severe clinical phenotypes, such as SPG44 and subclinical leukodystrophy [Abrams et al., 2014; Owczarek-Lipska et al., 2019]. These findings suggest that the *GJC2* mutations may lead to phenotypic heterogeneity and a spectrum of disorders [Biancheri et al., 2013].

Herein, we present a novel homozygous variant in the *GJC2* gene identified in an Iranian multi-affected family with phenotypic heterogeneity: HLD2 and hereditary spastic paraplegia (HSP) phenotypes. Finally, we review other *GJC2*-related neurodegenerative cases and describe the clinical and genetic findings of these patients. Our finding expands the mutation spectrum of *GJC2* and presents the first *GJC2*-related family with two distinct phenotypes in affected individuals.

## Subjects and Methods

This research was performed in accordance with the Declaration of Helsinki and with the approval of the ethics board of the University of Social Welfare and Rehabilitation Sciences (USWR; IR.USWR.REC.1401.038) in Iran. The participants were informed about the nature of the research and written informed consent was obtained for participation in this study.

*Subjects*

The subjects of the present study are members of an Iranian multi-affected family, HSP114 (Fig. 1a). They were referred to the Genetics Research Center (GRC) at the USWR for genetic analysis. The proband (III4) was initially diagnosed with HSP.

*Genetic Analysis*

Whole-Exome Sequencing

DNA of the proband was extracted from peripheral blood by the salting-out method. Exon enrichment was performed using the SureSelect V6-Post kit and whole-exome sequencing (WES) was done (paired-end) by an IlluminaHiSeq 2500 system (Illumina, CA, USA). WES analysis including sequence alignment of the proband against human reference genome UCSC NCBI37/hg19 using Burrows-Wheeler Aligner, duplicate read removal using the Picard, variant calling using Genome Analysis Toolkit, and annotation using ANNOVAR were implemented. Preliminary filtering was performed to identify all exonic, exonic splice, and splice site variants. Subsequently, filtering was done to identify all nonsynonymous homozygous or compound heterozygous variants. Finally, SNPs with a reported minor allele frequency of more than 0.01 in publicly available human variation databases were removed (online suppl. text S1.A). The remaining variants were examined to identify those within any of known HSP or other neurodegenerative disease-causing genes including amyotrophic lateral sclerosis, primary lateral sclerosis, leukodystrophies, mitochondrial and metabolic disorders, neurodegeneration with brain iron accumulation, ataxia, Parkinson's disease, and neuropathies causative genes.

Screening of the Candidate Variant

The candidate variant c.G14T:p.Ser5Ile in the *GJC2* gene was amplified from DNA of the proband by polymerase chain reaction. The polymerase chain reaction product was sequenced using the Sanger method (Big Dye kit and the Prism 3,130 sequencer; Applied Biosystems, Foster City, CA, USA). Sequence was analyzed using Sequencher 5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA) by comparison with the reference sequence available at NCBI: NC_000001.10, NM_020435.4 and NP_065168.2 for the *GJC2* gene. After confirming the variant in the proband, family members were also screened for co-segregation analysis of the variant (Fig. 1b).

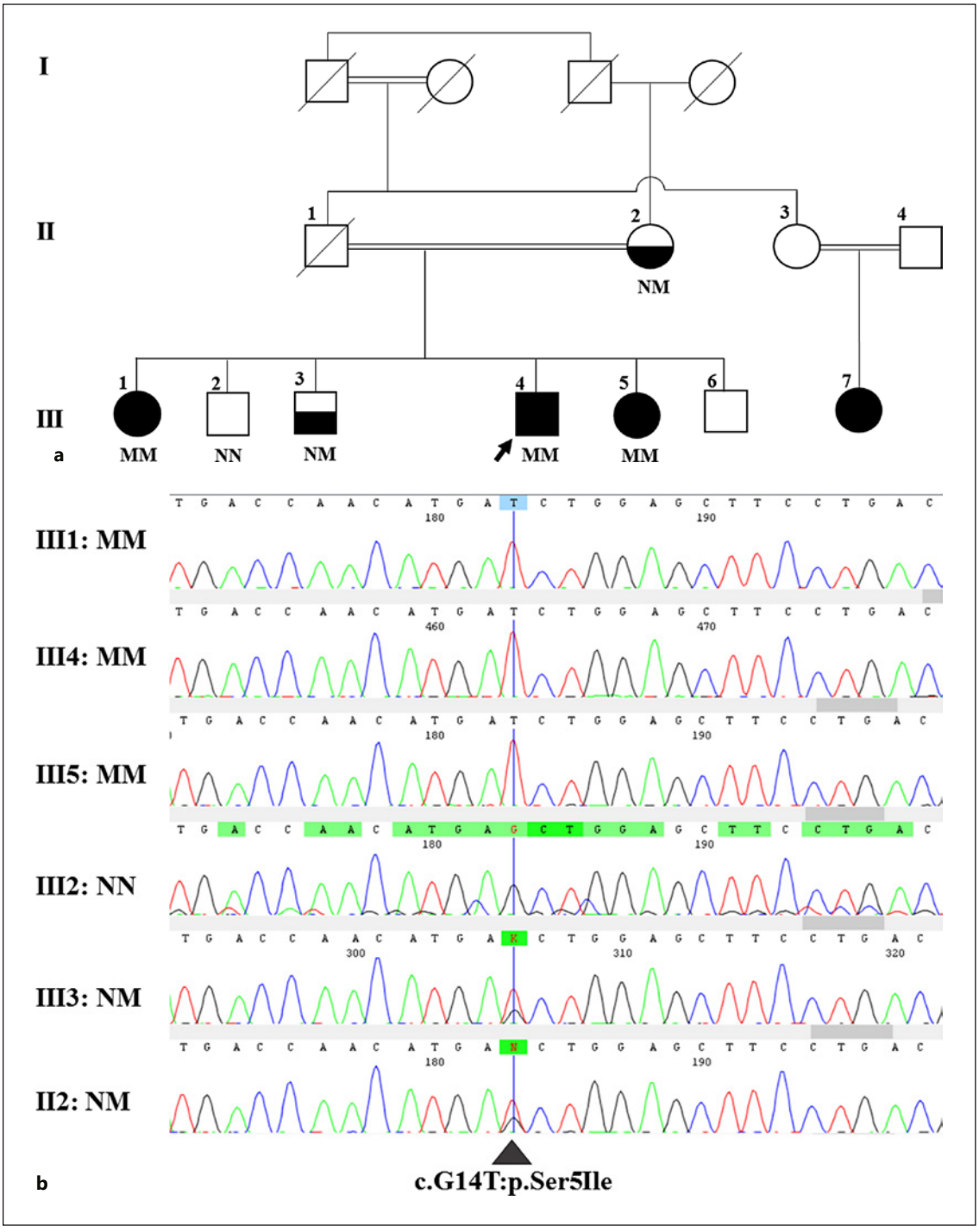



**Fig. 1. a** HSP114 pedigree with c.G14T:p.Ser5Ile variant in the *GJC2* gene. Genotypes of *GJC2* are shown when individuals were assessed. The arrow denotes the proband. Blank circles and squares: normal individuals; dark circles and squares: affected individuals; bottom filled symbol: heterozygous individual. **b** Sequence chromatograms of c.G14T:p.Ser5Ile variant in the *GJC2* gene in HSP114 family members. M, mutated allele; N, normal (wild type) allele.

In silico Analysis

The potential pathogenicity of the variant was evaluated based on the prediction scores of several in silico bioinformatics tools (online suppl. text S1.B). The variant was also assessed based on the American College of Medical Genetics (ACMG) criteria [Richards et al., 2015].

## Results

*Clinical Findings*

Totally, three affected and two unaffected siblings of HSP114 family, born to consanguineous parents, and their mother (Fig. 1a) were included in this study. The proband (III4) is a 38-year-old male (Fig. 1a). Since the age of 13, he began to show walking difficulties and leg stiffness which was more severe in right side. He also presented oscillopsia, urinary frequency, and tremor in his hands and head. He could walk independently for 37 years and is now unable to walk without assistance. On neurological examination, he had exaggerated jaw jerk reflex and there was side to side head tremor and postural and kinetic tremor in hands. He had truncal ataxia, impaired finger-to-nose test, and increased tone in his limbs (lower limb [LL] spasticity) accompanied by dystonic posturing. Deep tendon reflexes were increased with sustained ankle clonus and bilateral Babinski sign. Force of feet dorsiflexion was less than normal. He had a spastic gait (scissor gait). No cognitive problem was detected. He

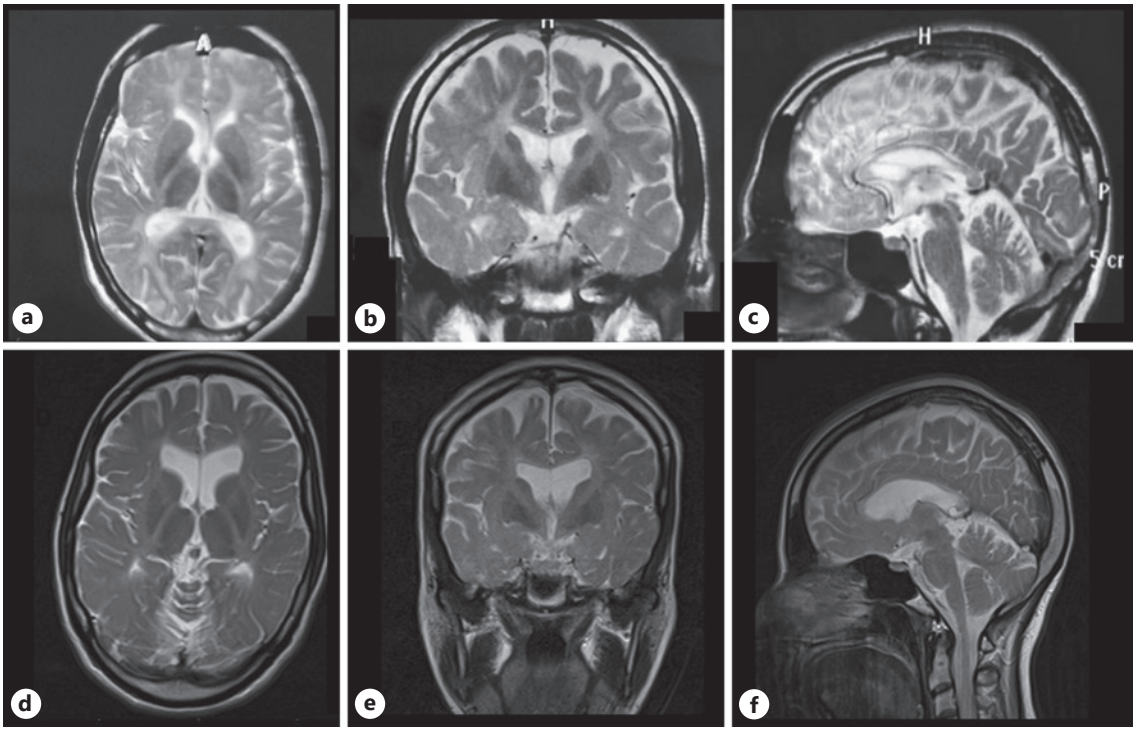
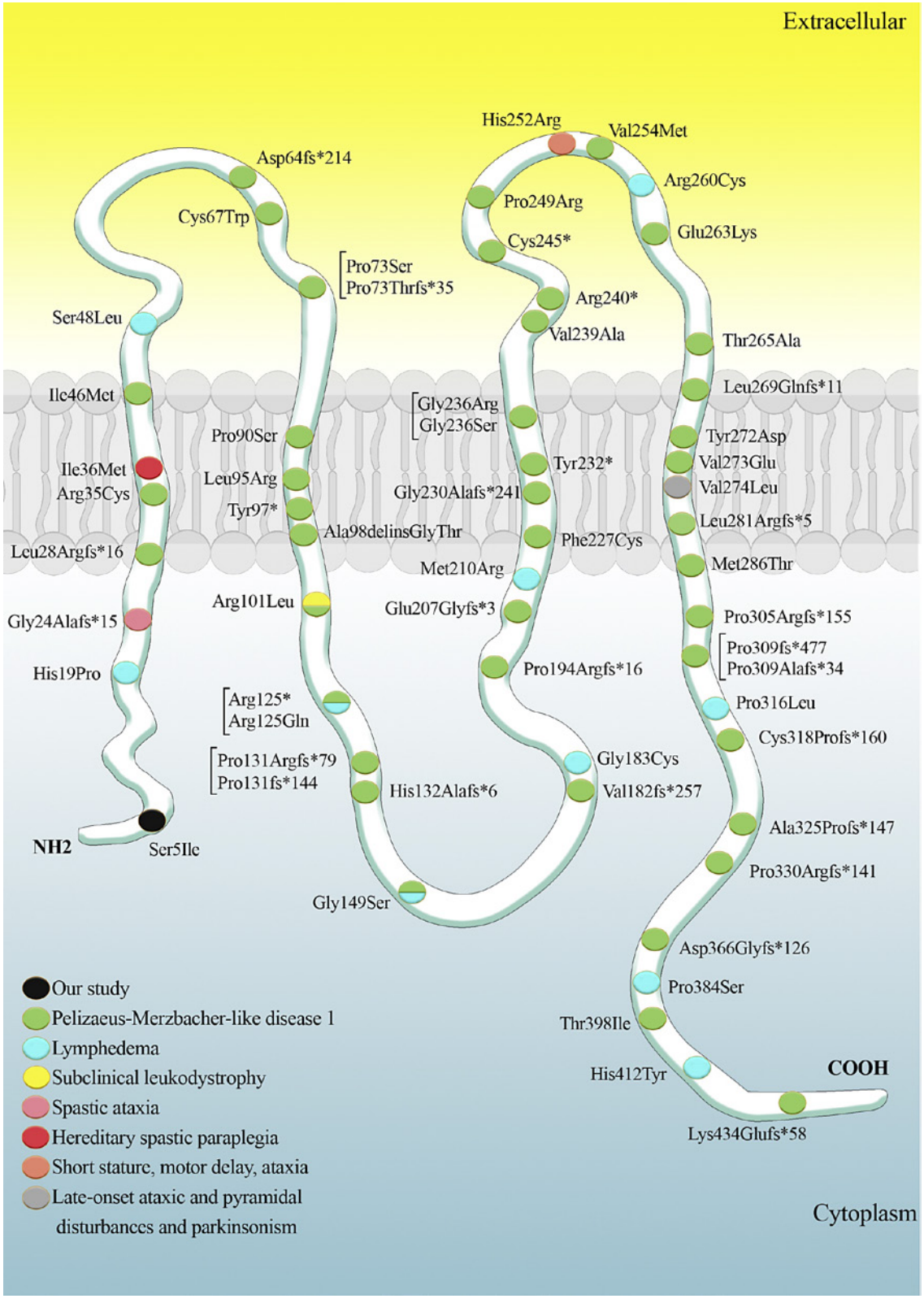



**Fig. 2.** Brain MRI of proband III4 (**a–c**) and case III1 (**d–f**) showing hypomyelination, cerebellar atrophy, and thin corpus callosum on T2 sequences.

**Fig. 3.** Schematic representation of mutations associated with *GJC2*-related phenotypes along the structure of connexin-47 (Cx47) protein. Cx47 protein is member of a large family of homologous connexins and comprises 4 transmembrane, 2 extracellular, and 3 cytoplasmic domains. Homozygous or compound heterozygous mutations in the *GJC2* gene are mostly responsible for hypomyelinating leukodystrophy 2 (HLD2), OMIM: 608804, or autosomal recessive Pelizaeus-Merzbacher-like disease-1 (PMLD1) (green circles). Several other diseases are also related to biallelic *GJC2* mutations such as hypomyelinating leukodystrophy (purple circles) and SPG44 (pink circle). Heterozygous missense mutations in this gene cause hereditary lymphedema (cyan circles). The zygosity of each variant (homozygous, heterozygous, and compound heterozygous) is not mentioned. See online supplementary Table S1 for more information. *(For figure see next page.)*

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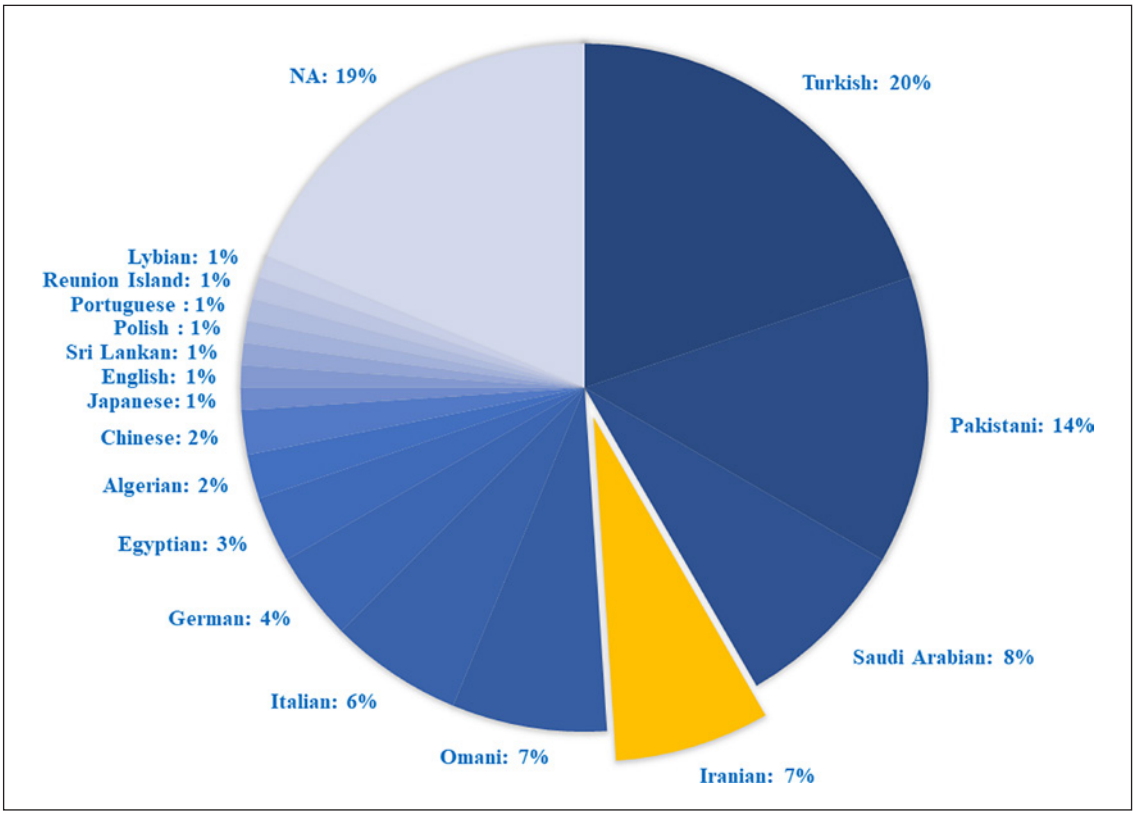


**Fig. 4.** Distribution of the homozygous/compound heterozygous *GJC2* mutations which are related to the neurological diseases in different populations, including our study. The most reported *GJC2*-related neurological patients were from Turkey (~20%), Pakistan (~14%), Saudi Arabia (~8%), Iran (~7%), and Oman (~7%).

had no nystagmus and fundoscopic examination revealed no optic atrophy. His brain magnetic resonance imaging (MRI) without gadolinium (Gad) showed diffuse bilateral symmetric cerebral white matter signal changes, mild hyper signal in T2-weighted and normal T1-weighted images compatible with hypomyelination leukodystrophy with hypomyelination of internal capsule, and brain stem in addition to thinning of corpus callosum, cerebellar atrophy, and relative preservation of the basal ganglia (Fig. 2a–c). His needle electromyography (EMG) and nerve conduction velocity (NCV) result was normal.

The proband's 42-year-old sister (III1) had several episodes of generalized tonic-clonic seizures at the age of 6 months and walking difficulties. She walked at 17 months. While she attended normal school, she was not able to complete high school because of gait problems. At the age 9 years, she underwent orthopedic surgery for contractures of the LLs. On neurologic examination, she had head titubation, scanning speech, bidirectional nystagmus, and increased jaw jerks. There was bilateral optic atrophy and she complained of visual problems. She had mild dystonic posture of hands, mild dysmetria on finger-to-nose test, spasticity of LL, increased muscle stretch reflexes, sustained ankle clonus, and bilateral Babinski sign. Presently, she cannot walk due to severe spasticity and contracture in LL. Sensory examination was normal including pinprick, pain, position, and vibration. No cognitive problem was detected. Her brain MRI without Gad revealed a very similar pattern of involvement to her brother including a hypomyelination pattern of extensive white matter involvement, thin corpus callosum, involvement of internal capsule, and mild cerebellar atrophy (Fig. 2d–f). Her EMG and NCV result was normal.

The proband's 36-year-old sister (III5) presented her first symptoms at the age of ~2 years with walking difficulties and mild leg stiffness. When she was 12 years old, her parents noticed a gait disturbance that gradually progressed to make her wheelchair-bound. On neurological examination, she had severe dysarthria, scanning, and spastic speech. There was bidirectional nystagmus and bi-

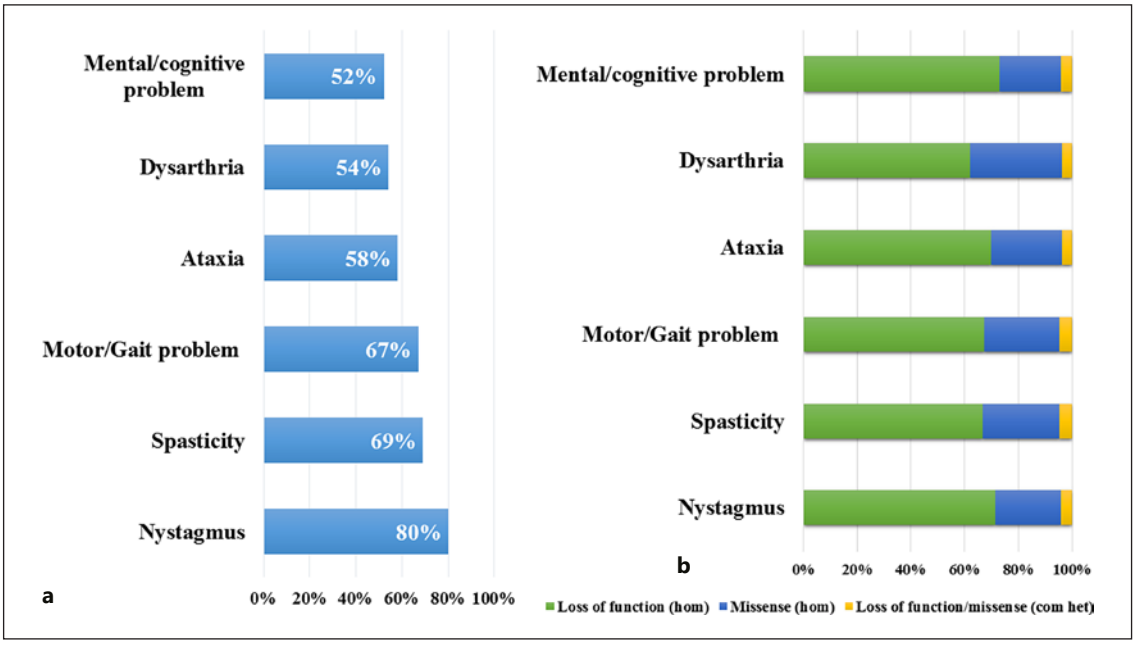


**Fig. 5. a** Typical features of *GJC2*-related neurological disorders. The typical features of *GJC2*-related neurological disorders are nystagmus as the first symptom and then spasticity, gait deterioration, ataxia, dysarthria, and mental/cognitive problem will be presented. **b** Typical features of *GJC2*-related neurological disorders according to the type of mutation. The typical features are often associated with loss-of-function mutations of *GJC2*.

lateral optic disc paleness on ophthalmoscopic examination. Tone of upper and LLs was spastic. On both sides, muscle stretch reflexes were increased and plantar reflexes were upward. The finger-to-nose test was impaired. She was not able to walk unaided. Her EMG and NCV result was normal. Her brain MRI was not available.

*Results of Genetic Analysis*

The preliminary filtering of WES data showed a novel homozygous variant, NM_020435, c.G14T:p.Ser5Ile, in the *GJC2* gene. The *GJC2* variant was absent in several exome/genome resources mentioned in the Methods section (online suppl. text S1.A) and co-segregated with the disease status in the family (Fig. 1b). The candidate variant was predicted to be damaging by variant by several in silico tools (online suppl. text S1.B) and uncertain significance variants by ACMG recommendations for variant classification.

## Discussion

To date, different neurological and non-neurological diseases associated with mutations in the *GJC2* gene have been identified (online suppl. Table S1; Fig. 3). Among *GJC2*-related neurological diseases, the distribution and frequency of mutations in this gene vary in different regions and ethnic groups (Fig. 4; online suppl. Table S1). These mutations are particularly prevalent in populations with a high rate of consanguineous marriages such as Turkey, Pakistan, Saudi Arabia, Iran, and Oman, which account for approximately 60% of all *GJC2*-related neurological cases (Fig. 4). Consanguineous marriages have been found in more than half of the *GJC2*-related neurological cases (~55%), including our study (online suppl. Table S1). A spectrum of *GJC2*-related neurological disorders exists, ranging from HLD2 to mild Cx47-associated diseases [Shimojima et al., 2013; Kuipers et al., 2019]. These phenotypes sometimes overlap with each other and it can be difficult to distinguish them [Zittel et al., 2012].

*GJC2* mutations are mostly associated with HLD2/Pelizaeus-Merzbacher-like disease-1 (Fig. 3; online suppl. Table S1) and found in about 8% of all PMLD patients [Shimojima et al., 2013]. In spite of variable expressivity, HLD2 patients have mostly similar clinical characteristics, with nystagmus as the first symptom as well as spasticity, gait deterioration, ataxia, dysarthria, and mental/cognitive problems (Fig. 5a). The typical features seem to be often associated with loss-of-function mutations of the *GJC2* gene (Fig. 5b). A typical disease onset age is 4.3

**Table 1.** Summary of clinical and paraclinical data of the HSP114 patients with the *GJC2* mutation

| Patient ID | III1 | III4[a] | III5 |
|---|---|---|---|
| Sex/age at examination, yr | F/42 | M/38 | F/36 |
| AAO | 6 mo | 13 yr | ~2 yr |
| First symptoms | Generalized tonic-clonic seizures | Walking difficulties and leg stiffness | Walking difficulties and leg stiffness |
| LL spasticity | + | + | + |
| UL spasticity | + | – | – |
| LL weakness | + | + | + |
| UL weakness | + | – | – |
| Gait problem | + | + | + |
| Babinsky sign | + | + | + |
| DTR | + | + | + |
| Clonus | + | + | + |
| Increased jaw jerk | + | + | + |
| Ataxia | + | + | + |
| Tremor | + | + | – |
| Dystonia | + | + | + |
| Dysarthria | + | – | + |
| Dysphagia | – | – | – |
| Seizure | + | – | – |
| Pes cavus | – | – | – |
| Cognitive problems | – | – | – |
| Nystagmus | + | – | + |
| Optic atrophy | + | – | + |
| Urinary problems | + | + | + |
| MRI | | | |
| Brain atrophy | + | + | NA |
| TCC | + | + | NA |
| Hypomyelination | + | + | NA |
| EMG | Normal | Normal | Normal |
| NCV | Normal | Normal | Normal |

yr, years; mo, months; LL, lower limb; UL, upper limb; DTR, deep tendon reflex; TCC, thin corpus callosum; M, male; F, female; AAO, age at onset; MRI, magnetic resonance imaging; NCV, nerve conduction velocity; EMG, electromyography. [a] Proband.

(±6.3) months in infancy (online suppl. Table S1) [Abrams and Scherer, 2012; Biancheri et al., 2013]. In comparison to "classical" PMD (Pelizaeus-Merzbacher disease) patients, HLD2 patients have a higher rate of peripheral neuropathy, seizures, and rapid neurological deterioration, along with partial myelination of corticospinal tracts. However, they exhibit higher motor and cognitive development [Uhlenberg et al., 2004; Yalcinkaya et al., 2012]. The higher intellectual level in HLD2 patients might indicate a more efficient myelination while a more rapid axonal degeneration in HLD2 may lead to loss of speech capacity before the age of 20 [Henneke et al., 2008].

Another form of *GJC2*-related neurological disorders is SPG44, autosomal recessive, a milder disorder than HLD2 that corresponds to milder changes on MRI. Interestingly, mutations in the *PLP1* gene are similarly responsible of both PMD, OMIM: 312080 (also termed leukodystrophy, hypomyelinating, 1), and spastic paraplegia 2, X-linked, OMIM: 312920 [Abrams, 2019]. So, it seems that there is association between PMD/PMLD-causing genes and HSP. SPG44 is a rare type of autosomal recessive HSPs which has been reported once, so far [Orthmann-Murphy et al., 2009]. Orthmann-Murphy et al. [2009] reported a large Italian family with three HSP-affected individuals. The patients manifested mild symptoms in the first or second decades of their life, and more severe progression with disability was observed in the third decade. Their physical examinations at ages 39, 36, and 53 years showed LL spasticity, spastic gait, extensor plantar responses, hyperreflexia, and pes cavus. Other features included dysarthria, loss of finger dexterity, dysmetria, and intention tremor on finger-to-nose and heel-to-knee testing. Nystagmus was not presented in the Ital-

ian cases. Their brain MRI showed a hypomyelinating leukodystrophy and thin corpus callosum in all 3 patients (online suppl. Table S1). Nerve conduction studies were normal in all 3 patients [Orthmann-Murphy et al., 2009].

There are some other mild neurological disorders associated with *GJC2* mutations [Zittel et al., 2012; Abrams et al., 2014; Kuipers et al., 2019]. For example, subclinical leukodystrophy, which results from a reduced efficiency of opening of Cx47 channels between oligodendrocytes and a preserved coupling between oligodendrocytes and astrocytes. The mild phenotype in these cases may be due to a partial loss of function of Cx47 channels, which has been reported once [Abrams et al., 2014].

Here, we described two distinct phenotypes – HSP and HLD2 – among affected individuals of a family who carried the same variant in the *GJC2* gene. The observed phenotypic heterogeneity in the patients of a given family with mutation in *GJC2* has not previously been reported. In this family, whereas the proband had a complicated form of HSP, both his affected sisters manifested HLD2. LL spasticity, hyperreflexia, pyramidal tract syndrome, dystonia, ataxia, and urinary problems were observed in all 3 patients while some typical presentations of HLD2 such as nystagmus, optic atrophy, and dysarthria were just observed in HLD2-affected sisters (Table 1). Also, unlike most HLD2 cases and both affected sisters, the proband presented the first symptoms at the age of 13 years. Almost all HLD2 patients typically present their symptoms in the first year of their life (online suppl. Table S1) [Abrams and Scherer, 2012; Biancheri et al., 2013]. The proband has been diagnosed with a complicated form of HSP based on clinical assessments by two specialists (A.T. and M.R.), whereas his sisters are affected by HLD2. Therefore, the proband III4 can be considered as a second report of SPG44. Nonetheless, Zittel et al. [2012] reported an atypical late-onset HLD2 patient with age at onset of 20 years who presented segmental dystonia and slowly progressive LL spasticity associated with mild hypomyelination on his brain MRI. Considering that the patient had a mutation in the *GJC2* gene, the authors suggested that this case of HLD2 mimicked the complicated HSP phenotype (online suppl. Table S1) [Zittel et al., 2012]. It seems, due to the late-onset form of disease and the absence of typical clinical findings of HLD2 such as nystagmus, this case could be classified as a rare *GJC2*-related HSP case than HLD2. As we consider this case, too, our proband becomes the third *GJC2*-related HSP case [Orthmann-Murphy et al., 2009] that shows the correlation between *GJC2* variants and HSP. Like our proband, all affected individuals of both reported families showed hypomyelination in their brain MRI [Orthmann-Murphy et al., 2009; Zittel et al., 2012] that suggests that hypomyelination may be a typical presentation in the brain MRI of SPG44 cases.

Cx47 protein which is encoded by the *GJC2* gene is a member of the protein family that forms gap junctions. Gap junctions are intercellular channels allowing electrical and metabolic communication between adjacent cells. 21 different connexins expressed in human tissues and their mutations have been associated with several diseases [Söhl and Willecke, 2004; Meyer et al., 2011]. Connexins are critical for normal myelination in the central nervous system [Menichella et al., 2003]. Astrocytes and oligodendrocytes are coupled by gap junctions and each cell type expresses different connexin proteins [Mar and Noetzel, 2008]. Gap junction communication and oligodendrocyte/astrocyte coupling is facilitated by Cx47/Cx43 heterotypic channels [Meyer et al., 2011]. Cx47 shares four transmembrane domains, which separate extracellular and cytoplasmic loop (Fig. 3) [Meyer et al., 2011]. The mutations of Cx47 that associated with the *GJC2*-related diseases have been spread throughout the protein and it seems there is no hot spot codon in this gene. Most of the reported *GJC2* mutations (~46%) are located in the cytoplasmic domain, while ~31% and ~23% of mutations occurred in the transmembrane and the extracellular domains, respectively (Fig. 3). Investigation of correlation between these mutations and their corresponding phenotypes revealed no obvious genotype-phenotype correlations in the *GJC2*-related neurological diseases. But it is postulated that almost all of the loss-of-function mutations including stop gain, frame shift, regulatory and splice site mutations result in the severe and early-onset form of *GJC2*-related neurological disease like HLD2, while usually milder phenotypes are caused by the missense mutations (online suppl. Table S1; Fig. 3). Of course, there are also the *GJC2* missense mutations that may result in the severe form of disease (Fig. 3). Or some mutations like p.Arg101Leu, in the homozygous or compound heterozygous state with another mutation, may be associated with the different forms of the disease such as subclinical leukodystrophy or HLD2 mimicking complicated HSP, respectively [Zittel et al., 2012; Abrams et al., 2014] (online suppl. Table S1; Fig. 3).

The severity of the disease may also be related to the location of the mutation as four mutations previously identified in patients with late-onset neurological features or sub-clinical phenotypes, including p.Ile36Met, p.Arg101Leu, p.Phe227Cys, and p.Val274Leu [Orthmann-Murphy et al., 2009; Zittel et al., 2012; Abrams et

al., 2014; Kuipers et al., 2019], are missense variants which are located within or very close to the transmembrane domains (online suppl. Table S1; Fig. 3). Nonetheless, our missense variant, p.Ser5Ile, affects amino acid 5 within the first cytoplasmic domain (Fig. 3) and associates with a spectrum of *GJC2*-related neurological disorders from mild to severe.

This study supports the notion that *GJC2* mutations may not always cause HLD2 disorders and also draws attention to phenotypic heterogeneity even within a family due to *GJC2* mutations. We suggest that screening of *GJC2* could be considered in patients with HSP or HSP-like phenotypes especially with hypomyelination in their brain MRI. This consideration should be highlighted especially in populations with a high rate of consanguineous marriages, such as those shown in Figure 4, because until now, the majority of reported *GJC2*-related cases have originated from Turkey, Pakistan, Saudi Arabia, Iran, and Oman. Identification of novel *GJC2* mutations and their related disorders may facilitate the identification of genotype-phenotype correlations, which may lead to a deeper understanding of pathophysiology of these disorders.


## Acknowledgments

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## Statement of Ethics

This research was performed in accordance with the Declaration of Helsinki and with the approval of the ethics board of the University of Social Welfare and Rehabilitation Sciences (USWR; IR.USWR.REC.1401.038) in Iran. The participants were informed about the nature of the research and written informed consent was obtained for participation in this study.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.


## Funding Sources

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## Author Contributions

Aida Ghasemi and Mana Khojasteh: DNA extraction, primer design, mutation screening of candidate variants, writing of manuscript, and analysis of exome sequencing data; Alireza Tavasoli: clinical evaluations and editing of manuscript; Mohammad Rohani: clinical evaluations and writing and editing of manuscript; and Afagh Alavi: designed and supervised the research and writing of the manuscript. All authors read and approved the final version of manuscript.

## Data Availability Statement

The data are available on request from the corresponding author.

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