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Short communication

Late-onset phenotype associated with a homozygous *GJC2* missense mutation in a Turkish family

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ABSTRACT

Objective: Recessive mutations in the *Gap Junction Protein Gamma 2* (*GJC2*) gene cause Pelizaeus-Merzbacher-like disease type 1, a severe infantile-onset hypomyelinating leukodystrophy. Milder, late-onset phenotypes including complicated spastic paraplegia in one family (SPG44), and mild tremor in one case, were reported associated to *GJC2* homozygous missense mutations. Here, we report a new family with two siblings carrying a different homozygous *GJC2* mutation, presenting with late-onset ataxic and pyramidal disturbances, and parkinsonism in one of them.

Methods: Two affected siblings were studied by neurological examination and brain MRI. Genetic analyses included genome-wide homozygosity mapping in both siblings, and whole exome sequencing in one sib. The resulting candidate gene variant was validated by Sanger sequencing.

Results: The affected siblings share a novel homozygous *GJC2* missense mutation (c.820G > C, p.Val274Leu), predicted as pathogenic by all used *in-silico* tools. Brain MRI showed hyperintense signal in T2-weighted images in the internal capsule and subcortical and periventricular white matter, consistent with hypomyelination.

Conclusions: Our findings confirm and further expand the late-onset phenotypes of *GJC2* mutations, to include prominent ataxia, pyramidal disturbances and mild parkinsonism, and confirm the distinctive associated MRI pattern.

1. Introduction

Connexins form gap junction channels that allow the exchange of small molecules via direct coupling between adjacent cells. The oligodendrocyte specific gap junction protein Connexin 47 (Cx47) is encoded by the *Gap Junction Protein Gamma 2* (*GJC2*) gene [1]. Over 30 recessive *GJC2* mutations (including some in the promoter regions) have been associated with neurological disorders, which usually present as Pelizaeus-Merzbacher-like disease (PMLD1), a severe infantile-onset hypomyelinating leukodystrophy [2]. A milder, late-onset phenotype of complicated spastic paraplegia has been reported so far in only one family with three affected individuals carrying a *GJC2* homozygous missense mutation, and termed SPG44 [3]. More recently, another homozygous *GJC2* mutation was reported in a subject with minimal clinical phenotype of slight postural tremor of the fingers by the age of 30 years-old [4]. Here, we broaden this phenotypic spectrum further by the identification of a new family from Turkey, carrying a new homozygous *GJC2* mutation, and manifesting with late-onset, slowly-progressive phenotype, mainly consisting of ataxic and pyramidal disturbances, and, in one patient, mild parkinsonism. The associated brain MRI pattern was consistent with hypomyelination.

2. Methods

We studied a Turkish family with two out of four siblings affected by a late-onset progressive neurological disease. Consanguinity was denied, but both parents originated from the same small village, suggesting a possibility of common ancestors. The study was approved by the local Medical Ethics Committee at the Istanbul Faculty of Medicine, Istanbul University, Turkey (protocol number 2014/705), and informed

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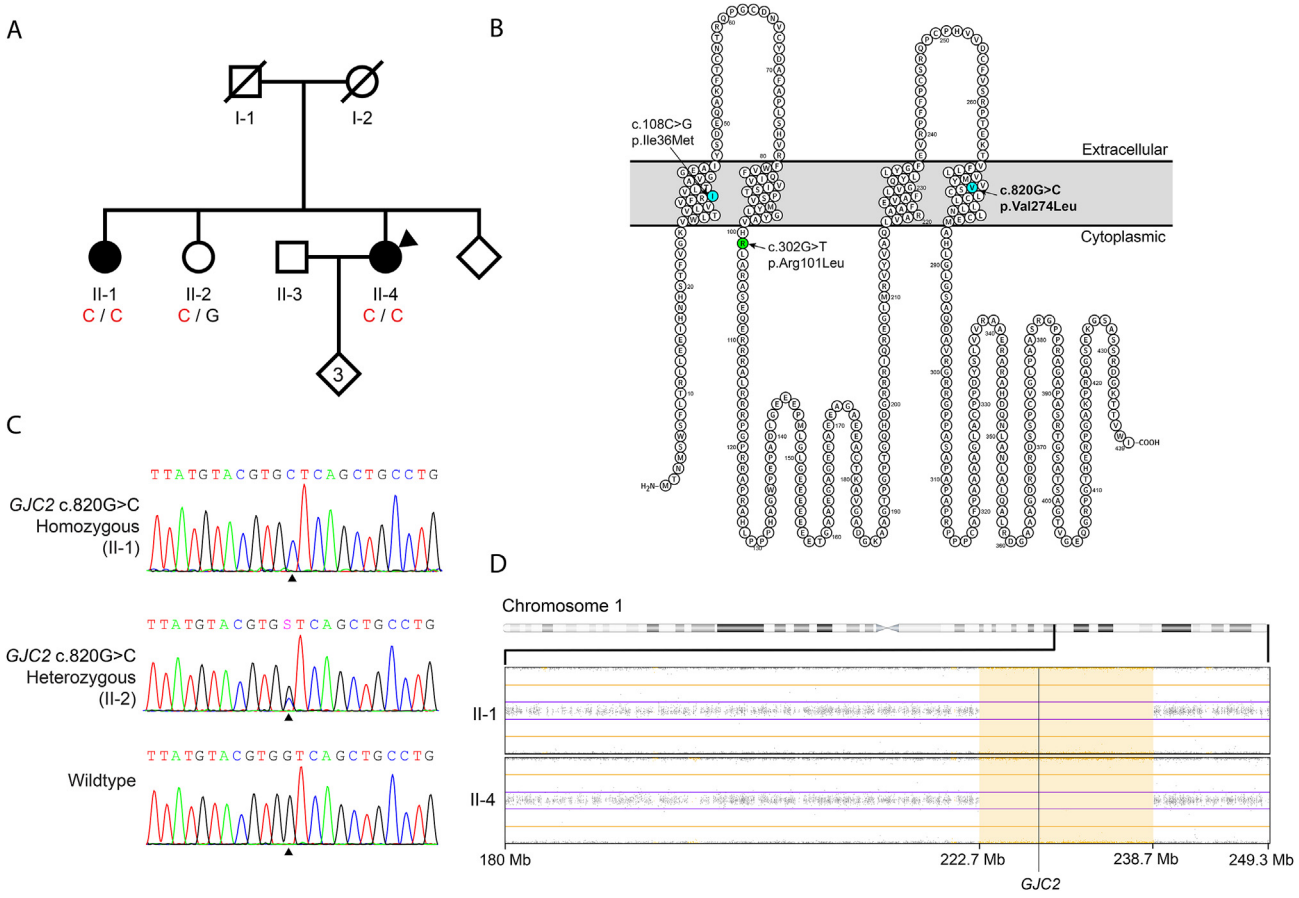

Fig. 1. Pedigree and results of genetic analyses.
(A) Family pedigree. Filled symbols denote affected individuals. The proband is indicated by the arrowhead. C, *GJC2* c.820G > C:p.Val274Leu mutation; G, wildtype *GJC2* allele. (B) Visualisation of the protein encoded by *GJC2* (Cx47) with variants found in this study and those reported previously in patients with late-onset phenotypes, previously annotated as c.99C > G, p.Ile33Met [3], and c.293G > T, p.Arg98Leu [4]. Variants are annotated in this figure according to GRCh37 transcript NM_020435.3. Location of the transmembrane domains was generated by the Protter tool (http://wlab.ethz.ch/protter).
(C) Electropherograms of one patient showing the homozygous *GJC2* mutation, one heterozygous carrier, and one unrelated control.
(D) Visualis
ation of the chromosome 1 homozygous region, spanning ~
16
Mb, shared by the two patients in this study.

consent was obtained from the participating subjects.

Genomic DNA of three family members (affected sisters II-1 and II-4, and their unaffected sister II-2, Fig. 1) was isolated from peripheral blood using standard protocols. Genome-wide single-nucleotide polymorphism (SNP) genotyping was performed using the HumanOmniExpress-12v1 BeadChip array (Illumina, San Diego, CA) in DNA samples from the subjects II-1 and II-4. Analysis of long runs of homozygosity was performed using GenomeStudio v.2011.1 (Illumina) and Nexus Copy Number (v.8, BioDiscovery, El Segundo, CA, USA). Whole exome sequencing (WES, mean read depth ~100X) was performed in the index patient (II-4) at the Centre for Biomics of the Erasmus MC, the Netherlands, using the SureSelect Human All Exon v5 capture kit (Agilent, Santa Clara, CA) and the Illumina HiSeq2500 sequencer, TruSeq v3 protocol (Illumina). The data were aligned to the human reference genome hg19 (GRCh37) with Burrows-Wheeler Aligner (BWA-MEM), and the NARWHAL pipeline [5,6]. Variant were called using the Genome Analysis Toolkit [7]. Due to the type of familial aggregation and origin of both parents from a very small village, we hypothesized an autosomal recessive mode of disease inheritance. Accordingly, we screened the WES data of patient II-4 for variants fulfilling the following criteria: (i) located within long runs of homozygosity; (ii) minor allele frequency ≤0.01 in the genome aggregation database (gnomAD r2.0.2; https://gnomad.broadinstitute.org/) and internal WES datasets; (iii) exonic nonsynonymous, or, located within 4bp from splice sites; and (iv) predicted to be damaging by the majority of *in-silico* tools (Appendix S1). We then performed Sanger sequencing of all the exons and exon-intron boundaries of the resulting candidate gene *GJC2* in the affected siblings (II-1, II-4) and unaffected sibling II-2. PCR primers and protocols are available in the supplementary data (Appendix S2).

3. Results

The index case (**II-4**) was admitted at 63 years old because of gait problems, frequent falls, and urinary incontinence. She reported normal motor and mental development and no history of epileptic seizures. Slight dizziness and imbalance were noted from the age of 37. These symptoms progressively worsened with unprovoked falls in the past 8 years. More recently she developed head tremor, cognitive problems, delusions, and impairment in activities of daily living. Upon neurological examination she displayed dysarthria, saccadic pursuits, and a positive glabellar tap sign. She had head tremor, and asymmetrical right-sided mild parkinsonism with rest and postural/kinetic tremor, mild rigidity and bradykinesia. She displayed frank appendicular and truncal cerebellar ataxia, and pyramidal signs (slight weakness in the bilateral iliopsoas muscles, brisk bilateral tendon reflexes, and Babinski sign). The neuropsychological evaluation revealed attentional deficits

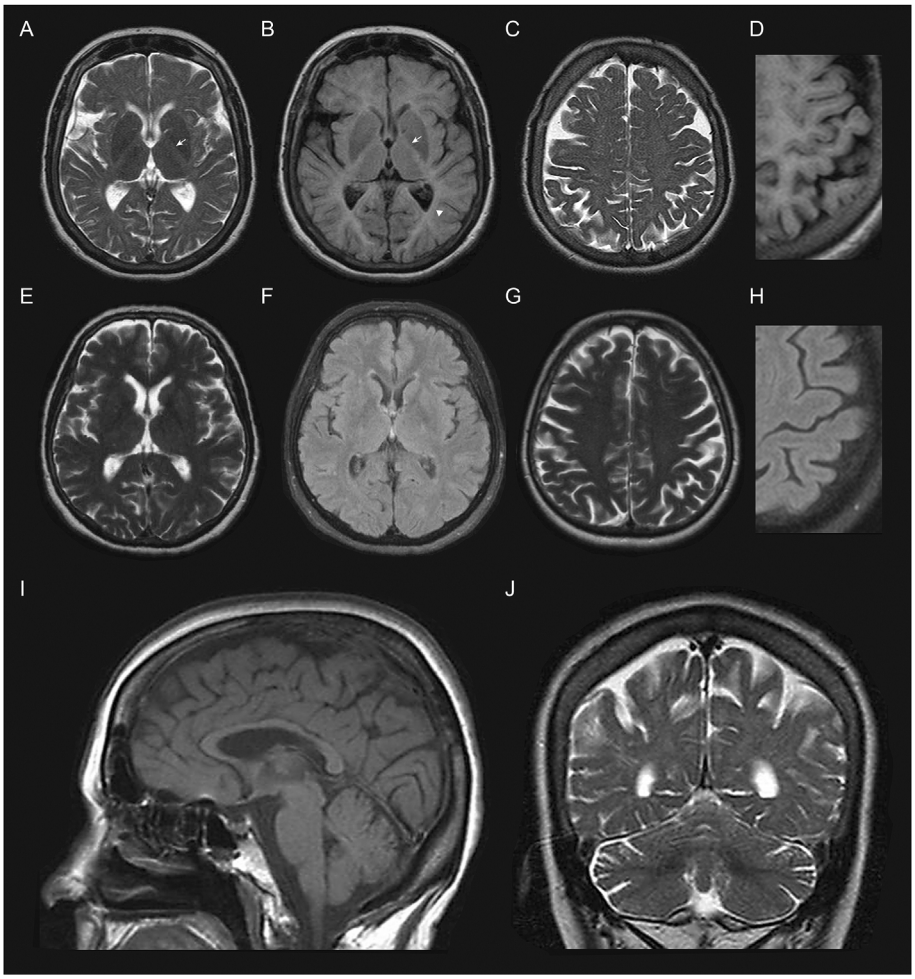

Fig. 2. Axial T2-weighted (**A, C**), T2 FLAIR (**B, D**), sagittal T1-weighted (**I**), and coronal T2-weighted (**J**) MRI scans of patient II-4 and corresponding MRI scans of an age-matched healthy control (**E-H**). The images show abnormal hyperintense T2 and T2 FLAIR signal in the posterior limb of the internal capsule (arrows in **A** and **B**), in the periventricular white matter (arrowhead in **B**), and in the subcortical, deep and periventricular white matter (**C**); a "pencil lining-like" signal results from the same type of signal abnormalities at the level of the cortical circumvolutions (**D**). Moderate cortical atrophy and diffuse thinning of the corpus callosum are shown on the sagittal T1-weighted image (**I**). The coronal T2-weighted image (**J**) demonstrates mild atrophy of the cerebellum and subtle cerebellar peripheral white matter changes compared to the more pronounced and diffuse white matter abnormalities in the supratentorial brain (**A-C**).

and dysexecutive syndrome with relative preservation of the other cognitive domains. Brain MRI revealed moderate frontal, temporal and parietal cortical atrophy, and abnormal diffuse hyperintense T2 and T2 FLAIR signal in the supratentorial white matter, including the posterior limb of the internal capsule, and the subcortical, deep and periventricular white matter (Fig. 2). Diffuse thinning of the corpus callosum was also present. The cerebellum showed mild atrophy and similar but milder signal alterations in the peripheral white matter.

The patient was initially screened for Wilson's and coeliac disease, which were negative. Her serum vitamin E, ceruloplasmin, and alpha-fetoprotein levels were normal. An electromyography study was normal, while a study of the visual evoked potentials showed a delayed P100 latency. Due to her parkinsonism symptoms she was prescribed levodopa and amantadine therapy, but showed no response to either treatment. Genetic analyses for spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA6, SCA7, SCA17), dentatorubral-pallidoluysian atrophy (DRPLA), and fragile X-associated tremor-ataxia syndrome (FXTAS), revealed no mutations.

The sister of the index case **(II-1)** also reported normal motor and mental development and no history of epileptic seizures. She reported tremor in the upper extremities starting around 15 years of age. She was examined at the age of 65 due to speech and gait problems, frequent falls, and urinary incontinence. These problems had started about 20 years earlier (at about 45 years of age), and caused impairment in the activities of daily living for the past 10 years. Her relatives noted that she exhibited behavioral disturbances such as hoarding and sleeping in public parks, and was scarcely collaborating with the medical staff. At our neurological examination, conducted during a home visit, the presence of a glabellar tap sign, dysarthria, and saccadic pursuits were noted. She displayed severe appendicular and truncal cerebellar ataxia, pyramidal signs, and tremor in her head and upper extremities (postural and intention, not at rest) without rigidity or bradykinesia.

Considering an autosomal recessive pattern of disease inheritance as the most likely, and the possible parental consanguinity, we hypothesized that a homozygous mutation was the cause of the disease in this family. Using SNP-array genotyping, we therefore searched for genomic regions that were homozygous and shared between the affected siblings (II-1 and II-4). This analysis identified five regions shared by the two patients and larger than 2 Mb in length (Appendix S3). We then used WES to identify candidate pathogenic variants within these regions of interest. This work yielded only one variant fulfilling our filtering criteria, a homozygous substitution in the *GJC2* gene (reference sequence NM_020435.3, c.820G > C, p.Val274Leu), located within the longest run of homozygosity (~16 Mb on chromosome 1) (Appendix S3). We also performed an unbiased, exome-wide variant filtering using the same criteria (but not limited to the homozygous regions), which revealed no additional candidate variants (Appendix S4).

The c.820G > C, p.Val274Leu variant is novel (not seen in the gnomAD database, comprising ~250.000 alleles), and all applied *in-silico* tools predicted a damaging (pathogenic) effect. Sanger sequencing confirmed this variant in homozygous state in the two affected siblings II-1 and II-4 and in heterozygous state in unaffected sibling II-2 (Fig. 1C).

4. Discussion

By genome-wide, unbiased homozygosity and WES analyses we identified a novel family with a homozygous *GJC2* pathogenic

mutation, expanding the late-onset clinical phenotypes of disorders linked to this gene and confirming its distinctive MRI pattern. These results illustrate the importance of unbiased genetic analysis in the differential diagnosis of rare inherited diseases with unspecific clinical presentations. We acknowledge that the lack of functional studies into the pathogenicity of the mutation is a limitation of this work. However, the identification of another mutation in the same gene in a family with an overlapping clinical phenotype and very similar MRI pattern by Orthmann-Murphy and colleagues [3], the predicted pathogenicity of the p.Val274Leu mutation across all *in-silico* tools utilized, its absence in any population databases, its segregation in homozygous state with disease in our family, and the absence of alternative candidate variants in the exome of our patient, all together provide strong evidence supporting our contention that the homozygous p.Val274Leu mutation is disease-causing.

The protein encoded by *GJC2* (Cx47) consists of 4 transmembrane, 2 extracellular, and 3 cytoplasmic regions (Fig. 1B). The p.Val274Leu variant is predicted to replace an amino acid within the last transmembrane domain, while the other two mutations previously identified in patients with late-onset or sub-clinical phenotypes [3,4] are located within the first and very close to the second transmembrane domain, respectively (Fig. 1B). We speculate that the p.Val274Leu and the other two previously reported mutations [3,4] induce only a mild or partial loss of the function of the Cx47 protein, thereby resulting in milder, late-onset phenotypes compared to the typical, infantile-onset PLMD1 presentations.

The clinical phenotype in the family reported here overlaps with that described for the SPG44 patients (Table S5) [3]. However, our patients display a predominant ataxic syndrome with additional pyramidal signs, urinary incontinence, and mild cognitive disturbances. Furthermore, one of our patients (II-4) showed unilateral parkinsonism with rest and postural/kinetic tremor, mild rigidity and bradykinesia.

Additionally, the onset age in our patients varied considerably, as II-4 suffered from dizziness and imbalance starting at 37 years of age and did not experience additional symptoms until her 6th decade of life. Her sister reported tremor in her upper extremities starting in her 2nd decade, but speech and gait problems did not appear until her 5th decade. The brain MRI abnormalities observed in our patients are strikingly similar to those in the patients reported previously with *GJC2* mutations and late-onset presentations [3,4], and consisting of abnormal white matter signal intensity in the posterior limb of the internal capsule, the subcortical, the deep and periventricular white matter, best visible as abnormal hyperintense T2 and T2 FLAIR signal of the involved white matter. This pattern is also typically seen in children with hypomyelinating leukodystrophies, including Pelizaeus-Merzbacher-like disease.

Of note, this abnormal MRI pattern should not be confused with that seen in some forms of neurodegeneration with brain iron accumulation, where the grey matter regions (such as the basal ganglia, thalamus, and cortical grey matter) appear hypointense in T2 MRI. Likewise, the images of the cerebral cortex on T2 FLAIR MRI (Fig. 2D) in our patient could resemble the 'pencil lining' sign reported in patients with neuroferritinopathy due to hypointense cortical grey matter [8], while in our patients the lining sign is caused by the hyperintense cortical white matter.

In conclusion, we report an unusual clinical phenotype of late-onset and slowly progressive multisystem degeneration with prominent cerebellar ataxia, pyramidal signs and, for the first time to our knowledge, also mild parkinsonism associated with a new homozygous *GJC2* mutation. We provide further evidence that mutations in this gene can cause milder and later-onset phenotypes and note the distinctive associated MRI pattern. This work has clinical relevance and screening of *GJC2* could be considered in patients with similar, otherwise unexplained phenotypes. The identification of additional patients with *GJC2* mutations might lead to a better appreciation of the associated late-onset phenotypes.

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Author roles

1) Research project: A. Conception, B. Organization, C. Execution.
2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique.
3) Manuscript Preparation: A. Writing of the first draft, B. Review and Critique.

DK 1BC, 2BC, 3AB
ZT 1BC, 2C, 3B
BB 1AB, 2C, 3B
SO 1BC, 2BC, 3B
MD 1C, 2C, 3B
WIJ 1C, 2C, 3B
GB 1C, 2C, 3B
GM 1B, 2C, 3B
HH 1A, 2C, 3B
ME 1A, 3B
VB 1AB, 2AC, 3AB

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.07.033.

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