

## Case report

Gordon Holmes syndrome due to compound heterozygosity of two new *PNPLA6* variants – A diagnostic challenge

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## ARTICLE INFO

## Keywords:

Gordon Holmes syndrome

*PNPLA6* gene

Hypogonadotropic hypogonadism

Ataxia

*FMR1* gene

## ABSTRACT

**Background:** Gordon Holmes syndrome (GHS), characterized by cerebellar ataxia and hypogonadotropic hypogonadism (HH), has been related to recessive mutations in *PNPLA6* gene.

**Aims of the study:** Describe one Portuguese family with GHS due to compound heterozygosity of two new *PNPLA6* variants.

**Methods:** Report on the clinical presentation, diagnostic and genetic workup to reach GHS diagnosis.

**Results:** The index case presented with slight cognitive impairment and primary amenorrhea, developed at the age of 25 a cerebellar syndrome. Her neurological exam revealed ataxia and mild extrapyramidal syndrome. She was born from non-consanguineous parents and had 8 siblings. Two of her sisters also had history of primary amenorrhea, tremor and ataxia. All 3 were diagnosed with HH and previous *FMR1* gene screening on her sisters revealed a 51 CGGs allele. However, 2 normal-sized *FMR1* alleles were identified on the proband thus excluding the FXTAS diagnosis in the family. Further *PNPLA6* variant screening revealed 2 novel variants in compound heterozygosity [c.2404G > C]; [c.4081C > T], which co-segregated with the disease.

**Conclusions:** This case shows how incomplete studies can be misleading, increases genetic knowledge of GHS and expands its clinical spectrum. The coexistence of a *FMR1* intermediate allele in this family constituted an additional challenge in the etiological investigation.

## 1. Introduction

Autosomal recessive cerebellar ataxias (ARCA) are a clinically and genetically heterogeneous group of neurodegenerative disorders often associated with additional non-cerebellar features [1]. Particular phenotypes can be found within this group, such as Gordon Holmes (GHS) and Boucher-Neuhäuser syndromes (BNS), in which ARCA appeared associated with hypogonadotropic hypogonadism (HH) [1,2].

Here we describe the clinical and genetic characterization of a family with GHS with 2 novel heterozygous *PNPLA6* variants.

## 2. Material &amp; methods

Detailed clinical, pedigree information and neurologic examination

were collected and performed, respectively. Imaging, blood and genetic studies completed all the data obtained.

Genomic DNA extraction was performed automatically on QIA Symphony platform, using the QIA Symphony DSP DNA Midi Kit. *FMR1* CGG allele sizing was performed by PCR and Southern blot analyses. Approximately 10 µg of gDNA was used for Southern blot analysis using GLFXD1 probe according to the manufacturer's instructions (GeneProber™, Gene Link, Hawthorne, NY). This method detects the *FMR1*-CGG repeat size as well as the methylation status. Targeted next-generation sequencing for *RNF216* and *PNPLA6* genes was performed, by multiplex-PCR amplification of all exons and flanking intronic regions using the Qiagen Multiplex PCR Kit, according to manufacturer's instructions. Next-generation sequencing (NGS) was performed using an Ion Torrent PGM™ platform, after ION Xpress™

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<https://doi.org/10.1016/j.ensci.2018.11.022>

Received 6 November 2018; Accepted 17 November 2018

Available online 22 November 2018

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Plus Fragment Library Kit workflow. Template and enrichment preparation was performed using the Ion PGM Hi-Q OT2 System. Sequencing reactions were carried out with the Ion PGM Hi-Q sequencing kit. Data analysis was performed using SeqNext (JSI™). PCR amplification and direct bidirectional Sanger sequencing of the exons harbouring the identified variants were applied to confirm the NGS data and for segregation analysis in the probands' family members.

This study was approved by the Institutional Review Board of Centro Hospitalar do Porto.

All investigations were performed with informed consent as part of a clinical diagnostic evaluation.

### 3. Results

#### 3.1. Clinical description

The proband (II-8) is a 46-year-old woman, with history of learning difficulties and primary amenorrhea. At the age of 25, she developed postural hand tremor and a progressive gait disorder with frequent falls. She remained autonomous in her daily and professional activities until the age of 44. She denied ophthalmological or other neurological symptoms.

On examination, she presented slight cognitive impairment, mild dysarthria, fragmented pursuit eye movements with normal saccades and horizontal and vertical gaze-evoked nystagmus. Slight generalized dystonia involving the cervical region (left laterocollis with intermittent negative tremor), right hand and left toes was present. She had an ataxic gait with highly impaired tandem gait. No other neurological or ophthalmological signs namely peripheral neuropathy or chorioretinal dystrophy were found.

Brain MRI showed severe cerebellar and vermis atrophy (Fig. 1). Laboratory tests, including complete blood count, renal, liver, thyroid function and vitamin E were normal/negative. The chromosomal analysis confirmed a normal female 46, XX karyotype. The basal hormonal evaluation revealed inappropriately low luteinizing hormone (LH) (1.3mUI/mL), as well as low follicular stimulating hormone (3.3mUI/mL), considering the level of estradiol (< 5 pg/mL). Human growth hormone was also low at < 0.05 ng/mL (0.06–10.00 ng/mL). Prolactin and cortisol levels were within normal range. There was no response to an intravenous dose of LH-releasing hormone, suggesting an abnormal pituitary function, consistent with HH. Pelvic echography revealed a hypoplastic uterus and the ovaries were not seen.

Family pedigree is represented in Fig. 2. No consanguinity was

identified. The proband's mother (I-1) is an 84-year-old woman without neurological problems. The proband's father (I-2) died at the age of 86 with dementia. Six siblings (II-5, 6, 7, 9, 10, 11) had learning difficulties and 3 sisters (II-9, 10, 11) developed primary amenorrhea, tremor and gait ataxia similar to the proband. One of the sisters (II-11) also had migraine and epilepsy. Another proband's sister (II-4) had an isolated typical dopa-responsive parkinsonism.

On twin sisters (II-10, 11) an intermediate *FMR1* allele, with 51 CGG, raised the possibility of fragile X tremor/ataxia syndrome (FXTAS) diagnosis. *FMR1* size mosaicism was excluded by Southern blot analysis. Although an intermediate *FMR1* allele was present in subjects I-1; II-3, II-4 and II-9, the index case showed normal sized alleles, so family-based studies made the FXTAS diagnosis unlikely. The II-9 sister was 45-year-old, had a mild intellectual disability, dysarthria, horizontal gaze-evoked nystagmus, left dysmetria, right arm dystonia and gait ataxia. Her brain MRI revealed cerebellar and vermis atrophy, similar to index patient, with slight cortical hypersignal and a small pituitary gland in accordance with HH (Fig. 3).

#### 3.2. Genetic analysis

Genetic screening by NGS allowed the identification of 2 variants in *PNPLA6*, in compound heterozygosity, both previously unreported. One was a missense variant, c.2404G > C; p.(Glu802Gln) and the other, a nonsense, c.4081C > T; p.(Arg1361\*) that results in a slightly shorter protein. The missense variant has not been reported in the literature, nor in ExAC/gnomAD or HGMD databases and is predicted to be disease-causing by all used in silico software, namely PolyPhen-2, SIFT, MutationTaster and UMD-Predictor. The familial investigation confirmed the segregation of the described phenotype with the variants identified in *PNPLA6*. No pathogenic variants were observed in *RNF216* gene.

### 4. Discussion

*PNPLA6* (patatin-like phospholipase domain-containing 6) gene variants can be responsible for a continuous spectrum of neurodegenerative disorders, from pure ataxia or hereditary spastic paraplegia to multisystem syndromes, such as GHS and BNS2. This broad clinical spectrum is likely to be related to the complex role of patatin-like domain within the brain, that is involved in lipid metabolism, neuronal development, intracellular membrane trafficking and axon maintenance [3]. Hufnagel et al. [4], discussed that also the timing and the

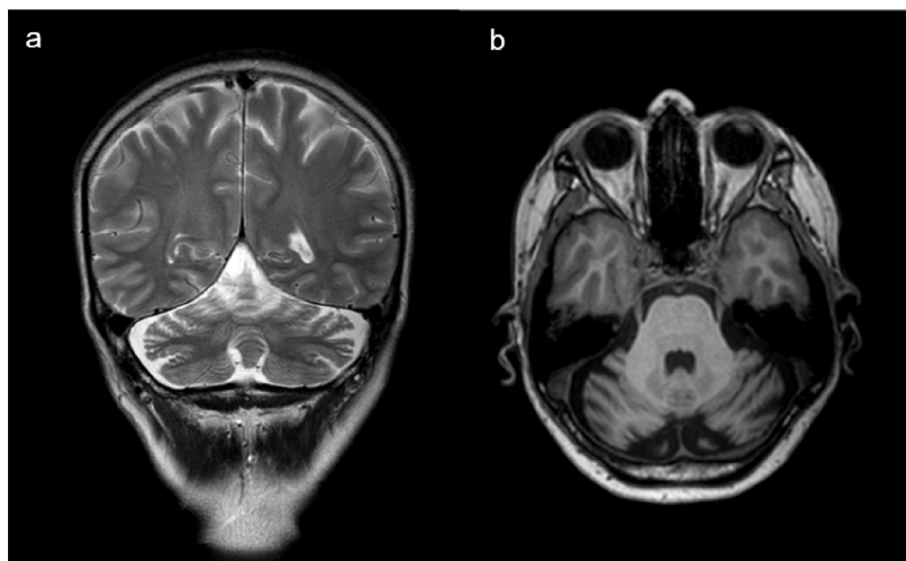
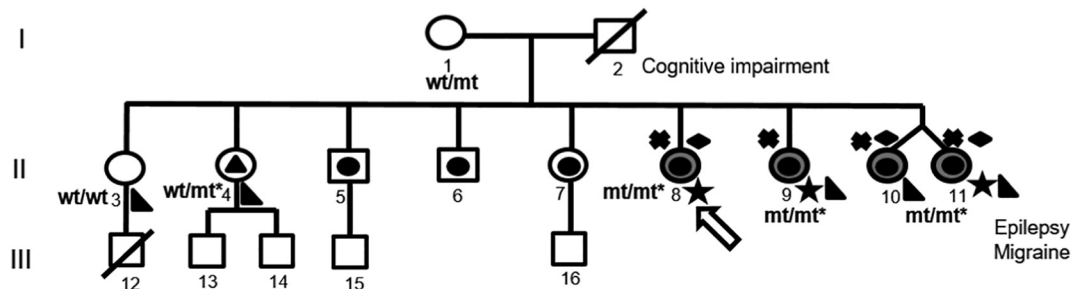
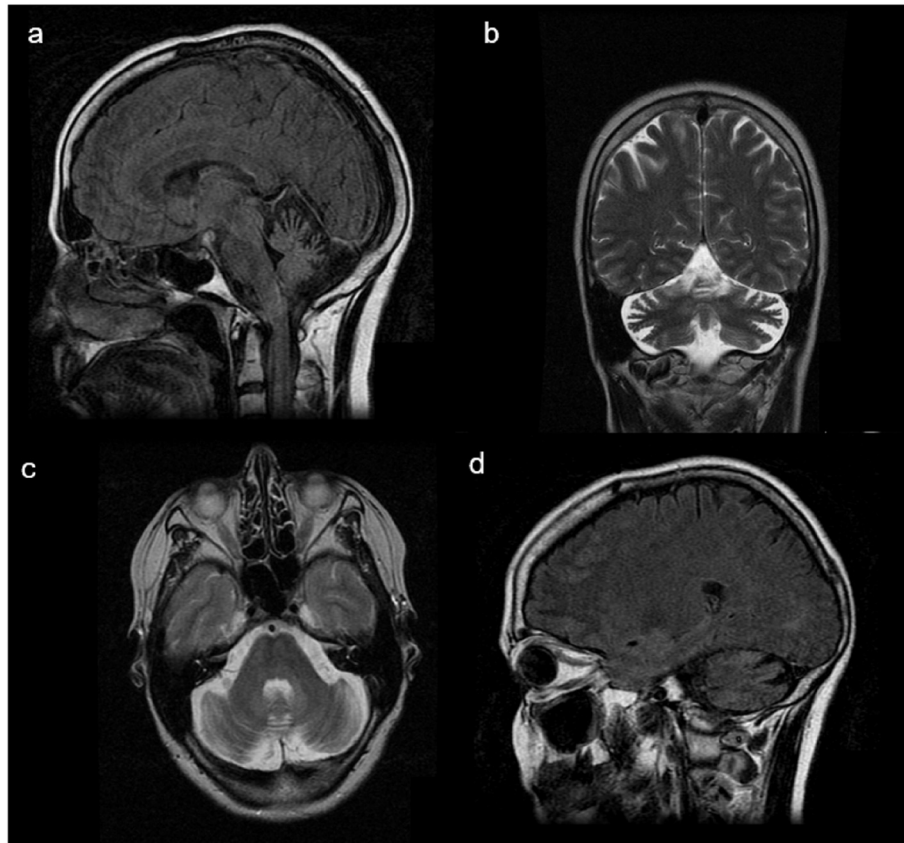


Fig. 1. Proband brain MRI showing severe cerebellar and vermis atrophy. Coronal T2- weighted (a) and Axial T1- weighted (b) sequences.



**Fig. 2.** The pedigree chart with segregation analysis of the variants identified in *PNPLA6* [the arrow denotes the index case, wt – wildtype, mt – c.2404G > C; p. (Glu802Gln), mt\* – c.4081C > T; p.(Arg1361\*), ▲ – intermediate allele for *FMR1*, ♦ – primary amenorrhea, ◆ – ataxia and tremor, ▲ – parkinsonian syndrome, ● – Intellectual disability, ★ – cerebellar atrophy].



**Fig. 3.** Proband's sister (II-9) brain MRI showing cerebellar and vermis atrophy (a-d), with a cortical hypersignal (c-d) and a small pituitary gland (a). Sagittal fluid-attenuated inversion recovery (a and d), coronal T2-weighted (b), axial T2-weighted (c) sequences.

severity of disease were related to the absolute hydrolase activity of the patatin-like domain and to unknown modifying factors within tissues or alternate enzymatic activities. In short, the deficiency of *PNPLA*-encoded neuropathy target esterase (NTE) may cause neurodegeneration, particularly affecting the cerebellum and reproductive function likely due to impaired gonadotropin release, which we call GHS [5].

We present a Portuguese family with GHS and compound heterozygosity for novel *PNPLA6* variants. GHS had an early-onset across all affected subjects, as expected [6]. In our opinion, the cognitive impairment could be considered the earliest manifestation of GHS, resulting in learning disability on several family members. Despite that, it was compatible with an active professional life until the forties. Cognitive impairment had been previously reported in patients with *PNPLA6* variants, with variable prevalence and severity [2,3,6].

Primary amenorrhea appeared as the first manifestation of HH. Later on, a progressive movement disorder developed, with onset

around the second-third decade of life, with ataxia and gait problems. Cerebellar atrophy, as we found in this family, is a frequent feature [6]. Interestingly, dystonia, epilepsy and migraine were also found in this family. Indeed, there are reports of focal dystonia and chorea in BNS [2,7], suggesting that the physiopathology of these diseases can include the extrapyramidal system, and epilepsy was suspected in 2 previous reported patients [8].

The identification of a *FMR1* intermediate allele in the family was a confounding factor and turned this diagnosis into a challenge. FXTAS is defined by CGG repeat expansions between 55 and 200 repeats [9] and alleles containing 45–54 repeats are considered gray-zone, with a clinical significance still to be established. However, recent reports showed that some individuals with intermediate alleles can develop FXTAS [10]. The coexistence of mental retardation, movement disorders (tremor, ataxia, parkinsonism) and infertility with a *FMR1* intermediate allele in some of the family members, made us consider

FXTAS as a diagnostic hypothesis. The identification of normal-sized *FMR1* alleles in the index case excluded it.

HH was the key to the diagnosis, making the multidisciplinary assessment with ophthalmologic, endocrine, genetic and neurologic evaluation crucial. Although without therapeutic consequences, the accurate diagnosis is very important to allow proper genetic counselling and surveillance programs with psychosocial support.

In conclusion, we present a family with GHS due to compound heterozygosity for novel *PNPLA6* variants. We considered cognitive impairment and dystonia part of the disease spectrum and speculate if they are related to this new *PNPLA6* variants. This family also raises awareness for the possibility of some correlation between GHS, epilepsy and migraine.

### Study funding

No targeted funding was received for this study

Unit for Multidisciplinary Research in Biomedicine – UMIB/ICBAS/UP is supported by National Funds through the FCT – Fundação para a Ciência e Tecnologia (Portuguese national funding agency for science, research and technology) in the frameworks of the UID/Multi/0215/2016 project.

### Declaration of interest

None.

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