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A Novel *RNF216* Variant Causing Gordon Holmes Syndrome in Three Lithuanian Siblings

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Abstract. ***Background:*** Gordon Holmes syndrome (GHS) is a rare genetic disorder, usually manifesting as a broad spectrum of neurological symptoms and hypogonadotropic hypogonadism. Only a limited number of cases presenting this congenital disorder have been reported in the literature. Due to the genetic and phenotypic heterogeneity of GHS, it is crucial to report novel cases.

Case presentation: Here we report a novel homozygous missense variant in the *RNF216* gene c.1055T>G (p.(Phe352Cys)) in three siblings. Primary concerns were the absence of secondary sexual characteristics, and amenorrhea occurred among female patients. Based on laboratory test results and clinical features, hypogonadotropic hypogonadism was diagnosed. Neurological examination revealed no signs of ataxia in the siblings. However, brain magnetic resonance imaging revealed pronounced changes in the cerebral white matter for the female patients. Due to primary amenorrhea and the absence of secondary sexual characteristics, treatment was initiated. Treatment might be adjusted in the presence of fertility considerations.

Conclusions: This case contributes to the limited knowledge of GHS and highlights the importance of hypogonadotropic hypogonadism treatment and close observation of neurological symptoms that may develop over time.

Keywords: Gordon Holmes syndrome, *RNF216*, hypogonadotropic hypogonadism.

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Naujas *RNF216* geno variantas, lemiantis Gordono–Holmso sindromą, nustatytas trims lietuvių šeimos vaikams

Santrauka. ***Įvadas:*** Gordono–Holmso sindromas (GHS) yra reta genetinė liga, dažniausiai siejama su plačiu neurologinių simptomų spektru ir hipogonadotropiniu hipogonadizmu. Literatūroje yra aprašyta nedaug šios įgimtos ligos atvejų. Todėl, atsižvelgiant į genetinį ir fenotipinį GHS heterogeniškumą, naujų klinikinių atvejų aprašymas yra itin svarbus.

Klinikinio atvejo pristatymas: Šiame straipsnyje aprašomas naujas homozigotinis *missense* tipo *RNF216* geno variantas c.1055T>G (p.(Phe352Cys)), kuris buvo nustatytas trims vaikams šeimoje. Pagrindinis pacientų nusiskundimas – nebuvo antrinių lytinių požymių, be to, moteriškosios lyties pacientėms buvo amenorėja. Remiantis laboratorinių tyrimų rezultatais ir klinikiniais požymiais, diagnozuotas hipogonadotropinis hipogonadizmas. Neurologinio ištyrimo metu ataksijos požymių pacientams nebuvo nustatyta. Tačiau galvos smegenų magnetinio rezonanso tomografijos vaizduose moteriškosios lyties pacienčių buvo matomi ryškūs galvos smegenų baltosios medžiagos pokyčiai. Buvo pradėtas gydymas dėl pirminės amenorėjos ir antrinių lytinių požymių nebuvimo. Ateityje gydymo taktika gali būti koreguojama, atsižvelgiant į vaisingumo poreikį.

Išvados: Šis klinikinis atvejis papildo ribotas žinias apie GHS ir pabrėžia hipogonadotropinio hipogonadizmo gydymo ir nuolatinio neurologinių simptomų, kurie gali išsivystyti laikui bėgant, stebėjimo svarbą.

Raktažodžiai: Gordono–Holmso sindromas, *RNF216*, hipogonadotropinis hipogonadizmas.

Background

Gordon Holmes syndrome (GHS; MIM #212840) is a rare, adult-onset neurodegenerative disorder, inherited in an autosomal recessive pattern. The spectrum of neurological manifestations is consistently associated with hypogonadotropic hypogonadism [1]. The syndrome was first described in 1908 by the British neurologist Gordon Holmes, who reported familial cerebellar degeneration accompanied by hypogonadotropic hypogonadism and ataxia [2]. The genetic basis of this complex neurodegenerative disorder is heterogeneous. Pathogenic variants in the *RNF216* (also known as *TRIAD3*), *PNPLA6*, *OTUD4*, and *STUB1* genes have been identified as key genetic determinants of GHS, involved in protein quality control and neuronal homeostasis [3]. *RNF216* encodes an E3 ubiquitin ligase that marks protein substrates for proteasome-mediated degradation, which is essential for autophagy, immune regulation, and synaptic plasticity. Disease-causing variations diminish this ligase activity and disrupt normal proteostasis, resulting in the accumulation of ubiquitin-positive intranuclear inclusions in the hippocampus and neuronal loss in both the cerebellum and hippocampus, as well as neuronal abnormalities in the hypothalamus and pituitary gland. Studies demonstrate that hypothalamic cells harbouring pathogenic variants in *RNF216* show a reduced gonadotropin-releasing hormone expression and diminished calcium signalling, leading to hypogonadotropic hypogonadism [4,5]. GHS is a rare disorder, the most recent literature review documented only 21 reported cases [6], with additional cases described subsequently [7]. Given the genetic and phenotypic heterogeneity of the syndrome, additional case descriptions are essential. The small number of published reports limits the understanding of the pathogenic significance and prognostic implications of genetic variants, as well as the full range of possible clinical phenotypes. We describe three siblings with clinical features consistent with GHS. The *RNF216* variant identified in this family has not been reported previously.

Case Presentation

The index patient (II:3) (Figure 1A) is a 22-year-old female with a history of primary amenorrhea. At the age of 17, the patient was consulted by a gynaecologist due to absent menses and poor development of breasts and pubic hair. An initial ultrasound examination revealed a uterus (size: 39 ×

21 × 44 mm) and ovaries (DO size: 28 × 15 mm; SO size: 24 × 19 mm); no follicles were visualized. Laboratory tests revealed a very low serum estradiol (E2) concentration (< 37 pmol/L, (reference range 77–921 pmol/l)), with inappropriately low luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels for the degree of hypoestrogenism (FSH 7.3 U/l (reference range 3.03–8.08 U/l), LH 3.12 U/l (reference range 1.80–11.78 U/l), and prolactin (PRL) 361.3 mU/L (reference range 108.7–557.1 mU/l). Due to low E2 concentration, inappropriately low FSH and LH levels, and poor development of secondary sexual characteristics, after excluding eating disorders and intense physical activity, hypogonadotropic hypogonadism was diagnosed. At the age of 17, the patient (II:3) was prescribed a combined oral contraception (ethinylestradiol/chlormadinone acetate), which induced withdrawal bleeding during use; menstruation ceased after discontinuation. A progesterone challenge (dydrogesterone) was negative on repeated gynaecologic evaluations, thus suggesting hypoestrogenism or endometrial hypo-responsiveness rather than anovulation alone. Subsequent hormone therapies were ineffective or poorly tolerated: estradiol/dydrogesterone did not induce bleeding, estradiol was discontinued due to intolerance, and a 3-month course of estradiol/norethisterone acetate did not restore spontaneous menstruation. The patient denies sexual activity. Estradiol/norethisterone acetate 2mg + 2mg/1mg + 1mg has been re-initiated. Since the age of 21, she has occasionally experienced dizziness, veering to the side while walking in the dark and a feeling of instability. On physical examination at the age of 22, her height was 163 cm, weight was 53 kg, and her body mass index was 19.9 kg/m^2. Head circumference was 54.5 cm. Minor findings included facial acne, a prominent philtrum, slight breast asymmetry, increased hair growth on the hands, and a café-au-lait macule approximately 2 cm in diameter on the right lumbar region. Neurological examination revealed no ataxia (SARA score was 0/40). Brain MRI revealed pronounced changes in the cerebral white matter (Figure 1B).

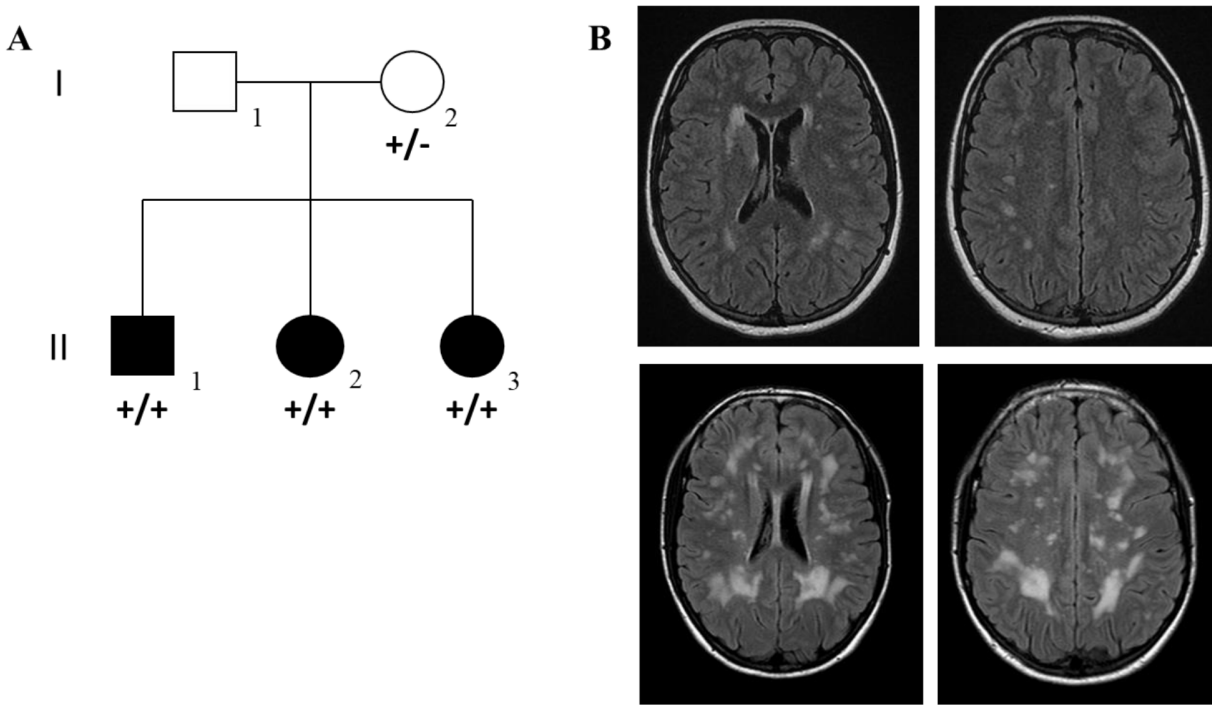

Figure 1. Pedigree of the family and neuroimaging. (A) Pedigree showing the affected siblings with GHS indicated by filled symbols, and *RNF216* genotypes denoted as +/+ for the homozygous c.1055T>G and +/– for the heterozygous c.1055T>G variant. (B) Brain magnetic resonance imaging of the affected siblings (II.2, aged 17 years (upper panel), and II.3, aged 22 years (lower panel)) showed multiple hypomyelinated lesions in the cerebral white matter (Ax T2 FLAIR)

Sibling (II:2) is currently 26 years old. She has reached an adult height of 157 cm. At the age of 14, the patient presented with primary amenorrhea, lack of secondary sexual maturation, including absent breast development, and pubic hair. Physical examination revealed clinodactyly of the second and fifth fingers. An initial ultrasound examination revealed a uterus (size: 29 × 16 × 25 mm) and ovaries (DO size: 19 × 10 mm; SO size: 18 × 9 mm). Laboratory tests revealed a very low serum E2 concentration (< 37 pmol/L (reference range 77–921 pmol/l)) and low levels of FSH (2.0 U/L (reference range 3.03–8.08 U/l)), LH (0.25 U/L (reference range 1.80–11.78 U/l)), and PRL (157.4 mU/L (reference range 108.7–557.1 mU/l)). The findings were consistent with hypogonadotropic hypogonadism after exclusion of secondary causes. Brain MRI was performed at the age of 17, revealing multiple, partially merged hypomyelinating lesions located in subcortical and periventricular regions in the white matter of the cerebral hemispheres (Figure 1B).

Additionally, the oldest sibling, 33 years old (II:1), is 168 cm tall, has no facial hair, and speaks with a high-pitched voice. The patient was diagnosed with hypogonadotropic hypogonadism in late adolescence. Neurological examination performed at age 33 revealed no signs of ataxia. No additional information for this patient was available at the time of genetic testing.

Genetic analysis

Karyotype analysis results were normal, 46,XX for both sisters (II:3; II:2) and 46,XY for the brother (II:1). Genomic DNA was extracted from peripheral blood leukocytes by using standard procedures. Exome sequencing and primary data processing were performed on a high-throughput next-generation sequencing platform (*Illumina, Inc*., San Diego, CA, USA) by *CeGaT GmbH*, Tübingen, Germany. The sequencing reads were aligned to the human reference genome (GRCh38/hg38), and variant calling was performed by using the *Illumina DRAGEN* platform. The average sequencing depth across the target regions was 100x. Variant annotation was performed by using an in-house bioinformatic pipeline incorporating ANNOVAR. Rare variants were filtered based on a quality score greater than 20 and a minor allele frequency below 0.02 in population databases as well as an internal database. Variants were prioritized according to their predicted functional impact, as assessed by using *in silico* tools, and their concordance with the phenotype. Variants were classified following the guidelines of the *American College of Medical Genetics and Genomics* (ACMG) and the *Association for Molecular Pathology* (AMP) [8]. A homozygous *RNF216* (NM_207111.4) gene variant c.1055T>G (p.(Phe352Cys)) was identified by next-generation sequencing analysis. The c.1055T>G variant is absent in public databases (ACMG criteria: PM2) and in the in-house database (PS4). *In silico* predictions using multiple tools indicate that this variant has a potentially damaging effect (PP3). Sanger sequencing confirmed that both affected siblings were homozygous for this variant (PP1; PP4), while their mother (I:2) was a heterozygous carrier, consistent with autosomal recessive inheritance (PM3). The alteration is located outside of RNF216 functional domains; however, the variant is within a functionally relevant region (Linear Ubiquitination – Associated E3 Ligases) of the *RNF216* protein annotated by the PANTHER database. The variant is evolutionarily conserved (GERP++_RS 5.72). Importantly, in earlier studies, reported pathogenic variants have been described in the same functional site of the *RNF216* gene, highlighting the functional significance of this region [9]. No additional candidate variants consistent with the phenotype were identified.

Discussion

We report a family with three siblings harbouring a novel homozygous *RNF216* variant. Affected individuals with biallelic variants in the *RNF216* exhibit decreased gonadotropin–releasing hormone expression and diminished calcium signalling, leading to hypogonadotropic hypogonadism [5]. The

majority of previously reported cases present with hypogonadotropic hypogonadism, followed later by dysarthria, ataxia, dementia with personality changes, and memory loss [4,6,10,11]. In line with previous reports, we report siblings presenting with hypogonadotropic hypogonadism. The most common manifestation of hypogonadotropic hypogonadism is absent or partial spontaneous puberty (48%). Other presentations include secondary amenorrhea, erectile dysfunction, and infertility [9]. In our presented case, hypogonadotropic hypogonadism manifested with primary amenorrhea for female patients (II:2; II:3) and absence of secondary sex characteristics for all siblings. Typically, absence of spontaneous or partial puberty in hypogonadotropic hypogonadism, related to the *RNF216* gene, is seen in males (63%), compared with females (13%) [12]. In the absence of secondary sexual characteristics, congenital hypogonadotropic hypogonadism (CHH) should be differentiated from constitutional delay of growth and puberty (CDGP) and primary gonadal defects. Primary gonadal defects were ruled out, since gonadotropin levels were not elevated. Both CDGP and CHH present with similar clinical features and hormone profiles in adolescents, resulting in difficult differentiation [12]. In the given case, genetic analysis was performed, revealing a *RNF216* gene variant associated with hypogonadotropic hypogonadism in all three siblings, excluding a CDGP diagnosis. Treatment to induce puberty is beneficial for sexual, bone, and metabolic health. It is recommended to treat hypogonadotropic hypogonadism with sex hormone replacement therapy for males and females [12,14]. The proband (II:3) received several different hormone replacement regimens and was eventually treated with Estradiol/Norethisterone acetate. Hormone replacement therapy is essential for patients with hypogonadotropic hypogonadism; however, it does not induce fertility. In congenital hypogonadotropic hypogonadism, infertility could be treated with gonadotropin-releasing hormone therapy [14]. In *RNF216*-related hypogonadotropic hypogonadism, it is unclear if restoration of gonadotropin secretion is sufficient to ensure fertility. Animal studies demonstrated that targeted deletion of *RNF216* disrupted spermatogenesis in mice, while female fertility was unaffected [15]. In addition, a female with a *RNF216* gene variant had two biological children [16]. Given the presented case, female patients (II:2; II:3) could achieve fertility; however, the fertility of the male patient (II:1) remains unclear.

Neurological manifestations typically begin before the age of 30. However, chorea and psychological symptoms represent an exception, as, in the majority of cases, their onset occurs after age 30. Ataxia and cognitive impairment may manifest before 30 years of age, after 30 years of age, or the age of onset may be unknown [9]. None of the siblings exhibited overt neurological manifestations at the time of evaluation, although the sibling (II:3) noted mild imbalance when walking in the dark. The neurological presentation in the reported patients appears attenuated and only partially expressed. Given the significant changes in the white matter, it could be considered to represent an early, developing stage of the neurological manifestations. Similar progression of neurological symptoms was observed in two male siblings with a homozygous missense variant in the gene *RNF216*. At the age of 28, one sibling first developed gait and speech disturbances. Three years later, he was severely ataxic. Another sibling, at the age of 27, exhibited mild cerebellar ataxia, which progressed two years later [11]. Existing evidence nevertheless suggests that frameshift variants are more often associated with more severe disease, including early-onset GHS and pronounced neurological involvement [6,17].

The majority of reported likely pathogenic variants were loss-of-function, and the most causative variants were usually located in the RBR domain or the C-terminal extension of *RNF216.* The published literature indicates that nonsense variants are the most prevalent (33%), followed by missense variants (25%) [6,9,17]. We report a novel missense variant (p.(Phe352Cys)), located outside the domains. According to the literature, genotype–phenotype correlations in GHS associated with *RNF216* variants remain limited. Different types of potentially pathogenic variants may lead to similar phenotypes, while identical variants can manifest with varying severity among individuals. Conclusions cannot be drawn without additional cases and functional studies.

The presented case has certain limitations. First, the interpretation of the identified variant is currently based primarily on *in-silico* tools and familial segregation analysis. Although these findings provide supportive evidence, functional validation is required to establish a definitive conclusion. Second, the small sample size further limits the generalizability of the findings.

Conclusions

We present a novel homozygous variant in the *RNF216* gene identified in three siblings, associated with GHS. This study contributes to the limited knowledge of phenotypic and genotypic variability in this rare disorder. It underscores the importance of long-term clinical follow-up, as neurological manifestations may emerge over time, and highlights the clinical relevance of timely recognition and management of hypogonadotropic hypogonadism.

Declarations of interest

The authors have no interest to declare.

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Author contributions

M. K.: conceptualization, data curation, visualization, writing – original draft preparation.
U. K.: conceptualization, data curation, visualization, writing – original draft preparation.
R. L.: conceptualization, investigation, resources, supervision, validation, writing – review and editing.
A. U.: supervision, validation, writing – review and editing.
B. B.: conceptualization, investigation, project administration, resources, supervision, validation, visualization, writing – review and editing.

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