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CASE REPORT

PHENOTYPIC AND MOLECULAR CHARACTERISTICS OF THREE ADDITIONAL PATIENTS WITH *HUWE1*-RELATED X-LINKED INTELLECTUAL DISABILITY

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ABSTRACT

Syndromic X-linked intellectual disability associated with pathogenic variants in *HUWE1* includes moderate to severe intellectual disability, dysmorphic features, and epilepsy, and is characterized by clinical variability, especially among carrier females. We present three unrelated symptomatic patients with severe neurodevelopmental symptoms and dysmorphic features, in whom molecular variants c.12469C>G, p.(Leu4157Val); c.5520+4_5520+7del, p.?; and c.4128G>A, p.(Met1376Ile) in the *HUWE1* gene were identified. Their phenotypic presentations were consistent with previously reported cases of pathogenic *HUWE1* variants. X-chromosome inactivation analysis in blood DNA revealed highly skewed inactivation (97:3) in a female patient, which may help explain the symptomatic course of the disease in her. De novo *HUWE1* pathogenic variants in females are frequently associated with a full-blown phenotype. In familial cases, carrier females may be asymptomatic or present mild cognitive impairment, while affected males often exhibit a more severe clinical course. Skewed X-chromosome inactivation may contribute to disease manifestation in female carriers.

Keywords: *HUWE1* gene, intellectual disability, X-chromosome inactivation

INTRODUCTION

Intellectual disability syndrome associated with pathogenic variants in the *HUWE1* gene, located on the X chromosome (Xp11.22) is characterized by global developmental delay with moderate to severe intellectual disability [1-4], absent or limited speech development, macrocephaly or microcephaly, and characteristic dysmorphic features such as deep-set eyes with epicanthic folds, hypotelorism, short palpebral fissures, dysplastic, large, or low-set ears, elongated face, bitemporal narrowing, high-arched palate, and thin upper lip. Other common features include hypotonia, epilepsy, scoliosis, delayed bone age, and short or tapered fingers. The condition follows an X-linked recessive inheritance pattern, with affected males and asymptomatic carrier females. In families with severely affected male's female carriers may exhibit mild neurodevelopmental symptoms or dysmorphic features. There are also documented cases of de novo *HUWE1* pathogenic variants in females leading to a full-blown phenotype, likely due to skewed X-chromosome inactivation [5-7].

The *HUWE1* gene encodes a ubiquitin E3 ligase responsible for substrate recognition in the ubiquitination process, a key post-translational modification. Ubiquitination modulates numerous cellular functions, including proteolysis, protein trafficking, signal transduction, enzymatic activity, chromatin remodelling, nuclear localization, and genome integrity. HUWE1 substrates are essential for maintaining cellular developmental homeostasis. Accordingly, HUWE1 is involved in cell proliferation and differentiation, apoptosis, DNA repair, and stress response. The HUWE1 protein contains several domains, with two mutational hotspots identified in the DUF908 and HECT domains, the latter being critical for the enzyme's ligase activity [8-10].

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Here we present three unrelated symptomatic patients—two females and one male—each exhibiting dysmorphic and neurodevelopmental phenotypes, including severe intellectual disability in the two older individuals. In all three cases, molecular variants in the *HUWE1* gene were identified. Written informed consent was obtained from all participants in accordance with institutional review board guidelines.

CASE PRESENTATION

Patient 1

A 16-year-old Polish girl was referred for genetic evaluation due to global developmental delay, including absent speech, severe intellectual disability, drug-resistant epilepsy, microcephaly, and dysmorphic features such as short palpebral fissures and epicanthal folds. Additional findings included tapered fingers and congenital anomalies: an atrial septal defect with mitral insufficiency and a submucosal cleft palate, which was surgically repaired at age 3. She was born as the first child to healthy, unrelated parents via cesarean section at 40 weeks of gestation, with a birth weight of 3000 g, length of 51 cm, and Apgar scores of 1–3–6–8. Developmental milestones were significantly delayed: sitting at 12 months, walking at 4 years, and speech limited to syllables.

Patient 2

A 9-year-old Albanian boy was referred for genetic evaluation due to intellectual disability, speech delay, reduced attention and concentration, hyperreflexia, hypotonia, and dysmorphic features including facial asymmetry, flat midface, long face, high forehead, downslanting palpebral fissures, a long philtrum with thin upper lip, depressed nasal bridge, micrognathia, protruding and posteriorly rotated ears, almond-shaped eyes, short and downslanting palpebral fissures, and thoracic asymmetry. Brain imaging revealed cerebral atrophy. He was the second child of healthy, unrelated parents from a twin pregnancy; the co-twin died in utero at 7 months of gestation. Birth weight was 4000 g, length 50 cm, and head circumference 37 cm. No epilepsy was observed.

Patient 3

A 3-year-old Albanian girl was referred for genetic evaluation due to global developmental delay, speech and language delay, reduced muscle mass with spastic tetraplegia, and optic atrophy. Additional findings included trigonocephaly, facial asymmetry, bifrontal narrowing, deep-set eyes, short and downslanting palpebral fissures, mild ptosis, and bilaterally elongated toes and thumbs. Following her first year of life, she developed seizures and EEG abnormalities, and antiepileptic treatment was initiated.

METHODS

The study was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants' parents. Molecular diagnostics were performed using genomic DNA automatically extracted from peripheral blood leukocytes.

Patient 1 underwent targeted sequencing using the TruSight One Sequencing Panel (Illumina, San Diego, CA, USA), followed by Sanger segregation analysis of the candidate *HUWE1* variant in the parents. Sequencing was performed on the HiSeq 1500 platform (Illumina) according to the manufacturer's protocol, with an average read depth of 130× and >95% of target regions covered at ≥20×. Reads were aligned to a modified GRCh38/hg38 genome assembly. Variant calling was performed using GATK HaplotypeCaller, MuTect2, FreeBayes, and DeepVariant. Copy-number variants (CNVs) were analysed with CNVkit and Decon. Variants were annotated using gnomAD v4.1.0 and evaluated using machine-learning meta-predictors (BayesDel, REVEL) and individual tools (AlphaMissense, CADD, EIGEN, FATHMM-MKL, MutationTaster, PolyPhen-2, SIFT). Variant novelty was assessed using ClinVar, LOVD, and HGMD. Interpretation followed ACMG guidelines. Variants were interpreted based on the guidelines of the American College of Medical Genetics. According to these criteria pathogenic and likely pathogenic (P/LP) variants were retained for analysis, while variants of uncertain significance (VUS) underwent more detailed clinical and genetic evaluation. Sanger sequencing was performed using the BigDye Terminator v3.1 Kit (Applied Biosystems) on an ABI 3130 Genetic Analyzer.

Patients 2 and 3 underwent whole-exome sequencing (WES) as trios. Genomic DNA was fragmented and target regions enriched using DNA capture probes covering >98% of coding RefSeq sequences (GRCh37/hg19). Libraries were sequenced on an Illumina platform to achieve ≥20× coverage for >98% of targeted bases. Reads were aligned to GRCh37/hg19 and the revised Cambridge Reference Sequence (rCRS) for mitochondrial DNA. Variants with MAF <1% in gnomAD or reported in HGMD, ClinVar, or CentoMD were evaluated. Variants are categorized into five classes (pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign) along ACMG guidelines for classification of variants. All relevant variants related to the phenotype of the patient are reported. CNV detection software had >95% sensitivity for deletions/duplications spanning ≥3 consecutive exons. *HUWE1* in Patient 3 was analysed by PCR and Sanger sequencing of the coding region and conserved exon-intron junctions.

The whole genome array CGH procedure (8x60K CGH) was performed following the manufacturer's in-

structions using Oligo/SNP array CGH (Agilent Technologies, Santa Clara, CA, USA). Arrays were scanned using a NimbleGen 200 Microarray Scanner (Roche Nimblegen, Madison, WI, USA). Feature extraction and data analysis were carried out with Agilent CytoGenomics 5.2.1.4 software (Agilent Technologies, Santa Clara, CA, USA) using default analysis settings. Genomic coordinates were interpreted based on the UCSC hg19 assembly. The nomenclature of reported molecular variants follows the Human Genome Variation Society (HGVS) guidelines.

X-chromosome inactivation (XCI) in patient 1 was analysed via the polymorphic CAG repeat in the androgen receptor gene (AR).

RESULTS

Patient 1

Initial genetic testing, including aCGH, showed no chromosomal abnormalities. Targeted NGS identified the known pathogenic variant in *HUWE1* gene: c.12469C>G, p.(Leu4157Val), confirmed by Sanger sequencing. Parental testing was negative, indicating a likely de novo origin. XCI analysis revealed a highly skewed inactivation (97:3).

Patient 2

Trio WES identified a novel hemizygous variant in *HUWE1* gene: c.5520+4_5520+7del, p.? currently classified as VUS. The asymptomatic mother is a carrier.

Patient 3

Trio WES a novel heterozygous variant in *HUWE1* gene :c.4128G>A, p.(Met1376Ile), absent in both parents, indicating likely de novo occurrence.

DISCUSSION

The phenotype of the presented patients, including neurodevelopmental symptoms and characteristic facial dysmorphia, is consistent with previously reported cases involving pathogenic variants in the *HUWE1* gene [5-7]. All three patients exhibited global developmental delay with absent or limited speech. Severe intellectual disability was diagnosed in patients 1 and 2. Patient 3 was only 3 years old at the time of assessment, and therefore her cognitive development requires further follow-up. Epilepsy and/or EEG abnormalities were observed in patients 1 and 3. Optic atrophy, seen in patient 3, has been previously described in single patient in literature. Additional findings, including atrial septal defect with mitral insufficiency and a submucosal cleft palate observed in patient 1, have not been reported in patients with *HUWE1I* variants. In the cohort described by Moortgat, several patients exhibited different genitourinary defects, such as hydronephrosis, cryptorchidism, shawl scrotum, or hypospadias [6]. None of patients reported here present such abnormalities.

The known de novo variant c.12469C>G, identified in patient 1, results in the missense substitution p.(Leu4157Val) within the HECT domain of HUWE1, which is critical for its enzymatic (ubiquitin-transferase) activity. This variant is absent from control databases including gnomAD (v4.1.0) and in-house databases (POLdb). Multiple *in silico* tools (REVEL, CADD, AlphaMissense, FATHMM-MKL, MutationTaster, SIFT) predict this substitution to be deleterious. It is reported as pathogenic in ClinVar (Variation ID: 375722) and has been classified as pathogenic according to ACMG guidelines [11]. A symptomatic course in this female patient is in line with previous reports linking de novo *HUWE1* variants to severe phenotypes in females.

In addition, X-chromosome inactivation analysis revealed a highly skewed pattern (97:3) in blood, likely favouring expression of the mutated allele. Similar patterns have been reported in the literature [6,7]. Skewed XCI in peripheral blood may suggest preferential inactivation of the wild-type allele; however, tissue-specific patterns remain unknown. If this skewing also occurs in neural tissue, preferential inactivation of the wild-type allele could contribute to the patient's phenotype. The molecular variant c.12469C>G (de novo) was reported in a symptomatic female patient by Moortgat [6]. Similar to our Patient 1, she had normal birth parameters, presented global psychomotor delay, absent speech, face dysmorphia, and epilepsy, but no cleft palate or cardiac defect. XCI analysis in that also indicated skewed pattern (100:0).

It should be noted that *HUWE1* variants c.5520+4_5520+7del and c.4128G>A (p.Met1376Ile), detected in patient 2 and 3 respectively, are classified as variants of uncertain significance (VUS) according to ACMG criteria. Pathogenicity predictions based solely on in silico analyses are insufficient to establish their pathogenic nature, particularly in the absence of functional studies. In Patients 2 and 3 other causative factors that may contribute to the phenotype, including genetic factors difficult to identify using the applied diagnostic methods, cannot be excluded.

The novel hemizygous variant c.5520+4_5520+7del, identified in patient 2, affects the splice donor site of intron 42, altering a non-conserved nucleotide. Splice prediction tools yielded conflicting results: MaxEntScan predicted a 36.6% reduction in splice site strength (suggesting exon skipping), while others (SpliceAI, SSF, NNSPLICE, Pangolin) indicated no significant impact. The variant was detected in 2 of 1,134,771 chromosomes in gnomAD (v4.1.0), with no homozygotes but one hemizygous occurrence. It was also reported in 4-years-old girl with delayed speech and attention deficit hyperactivity disorder (Centogen in-house report). If exon 42 is indeed skipped, this would result in a frameshift or premature stop codon and deletion of 119

amino acids, with possible impact on protein function. The variant is currently classified as a VUS (scoring 4 ACMG points: (PM2_supporting, PM4_supporting, PP3, PP4), with a calculated posterior probability of pathogenicity of 67.5%.

The novel heterozygous variant c.4128G>A; p.(Met1376Ile), identified in Patient 3, causes a missense substitution in the ubiquitin interaction motif (UIM). Both the affected nucleotide and amino acid are moderately conserved (phyloP = 5.81). Although methionine and isoleucine have similar physicochemical properties, *in silico* tools (CADD, AlphaMissense, FATHMM-MKL, Mutation Taster) suggest a deleterious effect. This variant was not found in gnomAD, the Exome Sequencing Project (ESP), 1000Genome, or POLdb. Missense variation in *HUWE1* is known to be associated with disease (missense Z-score = 11.82). The variant is currently classified as a VUS according to ACMG/AMP criteria (scoring 4 points: PM2_supporting, PM6_supporting, PP2, PP4), also with a 67.5% posterior probability of pathogenicity. Assuming the variant occurred de novo, as it was not found in either parent and considering that no other candidate variants were identified, it might contribute to phenotype of this patient. No other molecular variants affecting codon 1376 have been reported. The *HUWE1* variant located closest to this position, though in a different protein domain (WWE: tryptophan tryptophan glutamate), is c.3982A>G, p.(Met1376Ile), reported as de novo in a patient diagnosed with neutropenia, dysmorphia and intellectual disability. This variant was assessed as VUS according to ACMG criteria (scoring 4 points) with possible link to phenotype [12].

This report contributes additional clinical observations in patients with rare *HUWE1* variants. The phenotype of patients presented here is consistent with previously reported HUWE1-related ones, supporting a possible contribution of the identified variants; however, functional studies are required to confirm their pathogenicity. Neurodevelopmental disorders remain a diagnostic challenge despite increasing accessibility of large-scale sequencing techniques. The diagnostic yield remains modest, particularly in sporadic or complex cases. Testing for X-chromosome inactivation patterns in symptomatic female carriers of X-linked disorders may provide crucial insight into disease expression and support variant interpretation.

Conflict of interest statement

The authors declare no competing interest.

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