CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

Novel Genetic Variant in *HUWE1*

Prenatal and Postnatal Neuroimaging Phenotype

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Neurol Genet 2024;10:e200169. doi:10.1212/NXG.0000000000200169

Abstract

Objectives

To provide a comprehensive description of neuroradiologic findings in a patient with a probable pathogenic variant of *HUWE1*, particularly in relation to pontine and cerebellar hypoplasia.

Methods

We first report prenatal and postnatal neuroradiologic phenotype of a female patient carrying a *HUWE1* likely pathogenic variant and discuss its function.

Results

An ultrasound shows borderline ventriculomegaly, rotated cerebellar vermis, and dysgenetic corpus callosum. An MR study identify a short, thin corpus callosum, falcine sinus persistence, reduced cerebellar vermis size, wide inferior IV ventricle, and reduced pontine bulging.

Discussion

HUWE1 is a gene encoding an E3 ubitiquine ligase protein involved in nervous system development, function, and disease. The mechanisms by which *HUWE1* gene affects nervous system are still largely unclear, but a growing body of literature described disease-causing variants in this gene. This report may help prenatal diagnostic experts in consider also this entity, especially when dealing with pontine and cerebellar hypoplasia findings.

Introduction

HUWE1 is a gene encoding an E3 ubitiquine ligase protein, involved in cell growth and death regulation, tumor development, DNA damage response, CNS function, and inflammosome activation.[1-3] Gene copy number variants and missense mutations have been reported in individuals with syndromic and nonsyndromic X-linked intellectual disability, possibly associated with autism, and can cause minor dysmorphic CNS or facial features, detected through MRI.[3-6] Most patients previously described have no available neuroimaging data, and most who underwent brain MR imaging had a normal examination.[5,7]

We report a fetal and postnatal case in which a novel genetic variant in *HUWE1* gene was identified (c. 273 G>C, p.Met91Ile), aiding prenatal diagnostic experts in recognizing this entity, particularly in cases of pontine and cerebellar hypoplasia.

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Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

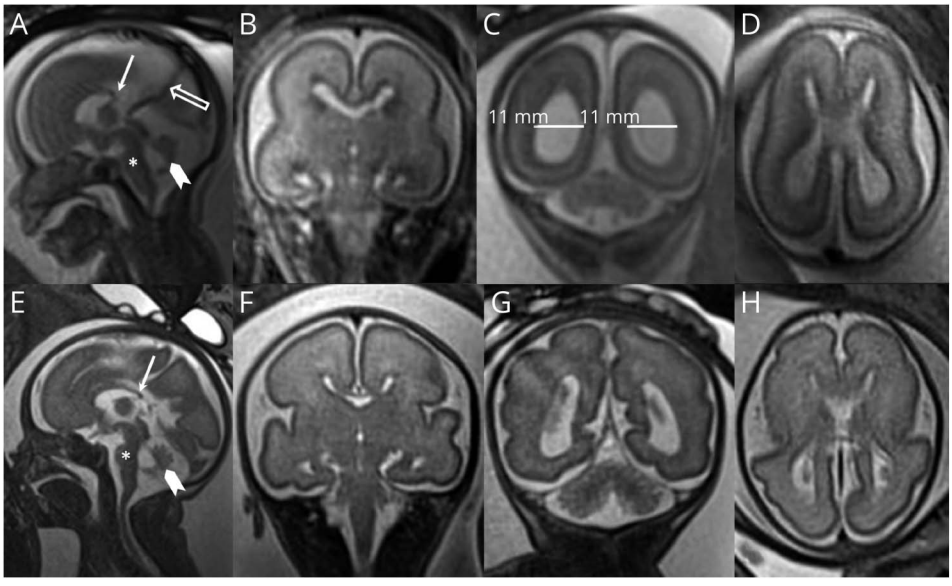
The Article Processing Charge was funded by the authors.

Case Description

A 43-year-old woman with 3 previous abortions, no consanguinity, no perinatal infections, or known teratogenic risk medications was referred to our maternal fetal unit. An ultrasound examination at 20 weeks showed borderline ventriculomegaly, rotated and hypoplastic cerebellar vermis and dysgenetic corpus callosum. Fetal MRI at 21 weeks identified a short and thin corpus callosum with mild ventriculomegaly (atrium: 11 mm), falcine sinus persistence; cerebellar vermis was reduced in size compared with normal parameters (superior-inferior diameter: 7 mm vs 8 mm; anterior-posterior diameter: 4 mm vs 5 mm); inferior part of the IV ventricle was wide because of mild cranial rotation of the vermis; reduced pontine bulging was evident too[8] (Figures 1 and 2). NGS panel genes related to prenatal phenotype (cerebellar hypoplasia and corpus callosum hypoplasia) was performed on genomic DNA by using the Twist Custom Panel Kit clinical exome—Twist Bioscience according to the manufacture's protocol on an NovaSeq6000 platform (Illumina). The reads were aligned to human genome build GRCh37/UCSC hg19. The BaseSpace pipeline and the Geneyx software (LifeMap Sciences, Inc.) were used for the variants' calling and annotating, respectively. Putative disease-associated variants were filtered for an allele frequency <1% in Genome Aggregation Database (gnomAD) and were checked based on the public databases (dbSNP), Exome Aggregation Consortium (ExAC) and gnomAD. The variants were evaluated by VarSome and categorized in accordance with the ACMG recommendations.[9] Variants were examined for a minimum depth coverage of 30X and Qscore (minimum threshold of 30) and visualized by the Integrative Genome Viewer. NGS-based trio test allowed to identify a novel de novo heterozygous missense variant c.273G>C (p.Met911Ile) in the *HUWE1* gene (NM_031407) (eFigure 1). The missense change is not present in the reference population database (dbSNP), ExAC, and gnomAD and is not previously described in literature. It involves a highly conserved residue across species and is predicted to be deleterious (scaled CADD score 21.70) and can be classified a likely pathogenic variant (class 4 according to the ACMG criteria). We did not perform the X inactivation study on the amniocytes. The couple opted for continuation of pregnancy. Fetal MR imaging, repeated at 28 weeks, revealed dysmorphic ventricular system because of the abnormal corpus callosum, thin and short. Biometric parameters of the cerebellar vermis, cranially rotated, appeared at lower limits for GA (SI diameter: 13 mm; AP diameter: 9 mm). Reduced pontine bulging was detected with smaller cranio-caudal diameter of the pons (Figure 1). The III-trimester of gestation was also complicated by severe polyhydramnios which required amniotic fluid drainage.

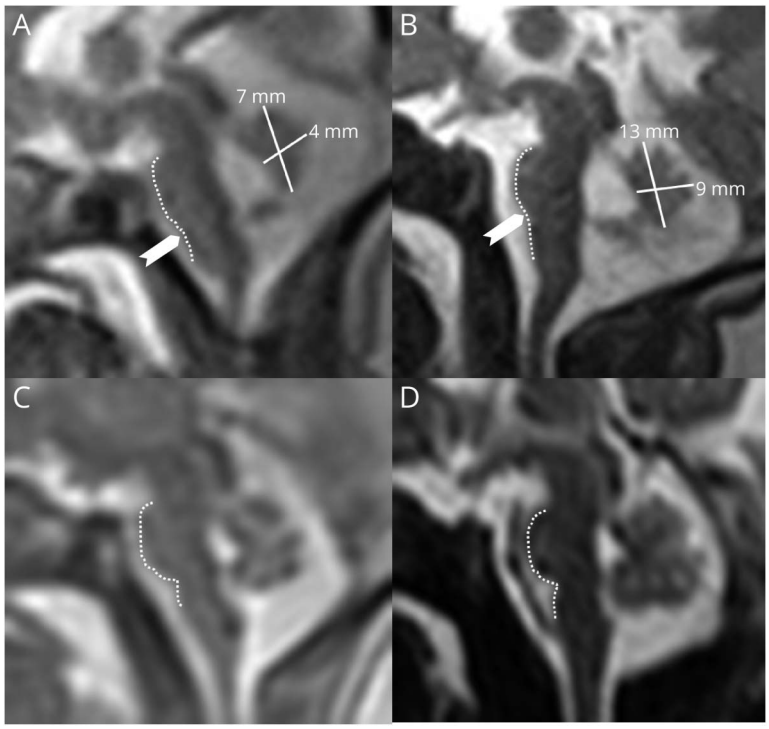
The patient was born at 38 + 1 weeks by elective cesarean section. Her birth parameters were as follows: birth weight 3,240 g (72° centile); length 47 cm (22° centile); occipitofrontal circumference 32 cm (12° centile). Apgar was 6/8 at 1st minute and 5th minute. Mild dysmorphic features were observed (thin upper lip, deep set eyes, and epicanthic folds). Neonatal MR imaging, at 4 days of life, confirmed pontocerebellar hypoplasia with significant cranial rotation of the cerebellar vermis and medulla oblongata gross appearance, reduced evidence of anterior fissure, dysgenesis of corpus callosum with reduction of AP diameter, and thickness of the medial and posterior portions. In addition, an occipital median skull defect at the level of tentorium insertion as an intracranial lipoma (Figure 2). At birth, heart ultrasound showed pulmonary hypertension with an estimated pulmonary artery pressure of 60–65 mm Hg. At 1 month, pulmonary pressure had turned

Figure 1 Supratentorial Abnormalities in Fetal MRI



Midsagittal balance (A, E), coronal (B, C, F, G), and axial (D, H) T2-weighted Ssh sections from 21-week and 28-week prenatal study, respectively, showing thin and short corpus callosum (arrows), lateral ventricles dysmorphism, smaller and partially upward rotated cerebellar vermis (arrowheads), reduced pontine bulging (asterisks), persistent falcine sinus (empty arrow), and mild ventriculomegaly only in the first examination.

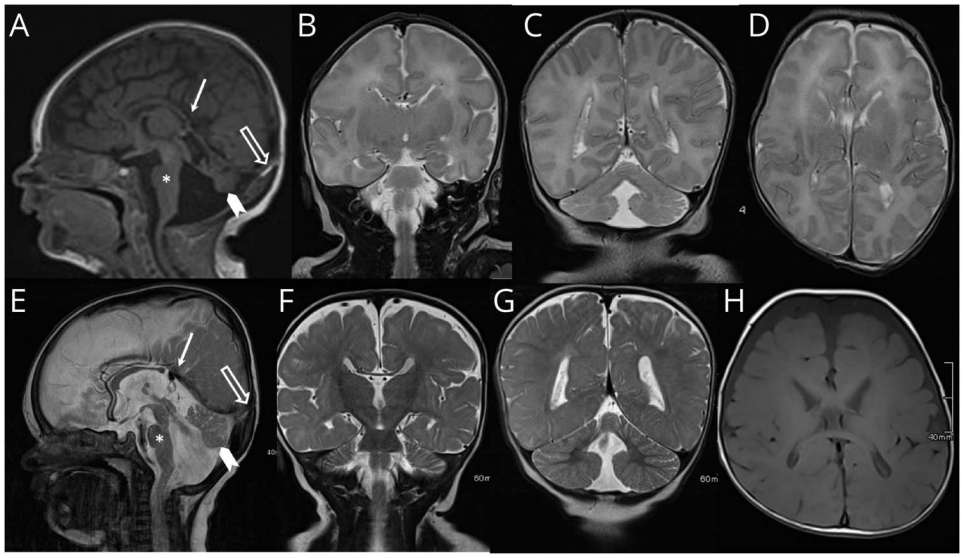
Figure 2 Infratentorial Abnormalities in Fetal MRI



Midsagittal T2-weighted Ssh sections of our patient from 21-week (A) and 28-week (B) prenatal study showing smaller and partially upward rotated cerebellar vermis, reduced pontine bulging (dashed line), gross appearance of the medulla oblongata with poor representation of the groove at the border of the pons. Midsagittal T2-weighted Ssh sections of a normal fetus from 21-week (C) and 28-week (D) prenatal study showing, by comparison, normal appearance of the structures of the posterior cranial fossa.

into normal parameters. Auditory brainstem response was normal, while ophthalmologic evaluation showed a mild atrophy of optic papilla. The child developed hyperopia and strabismus at 6 months. While neurologic evaluation at birth was normal, the neurologic follow-up revealed hypotonia and neurodevelopmental impairment. She started crawling at 15 months and began walking with support at 20 months. No seizures were reported, and subsequent EEG did not show any epileptic anomalies. Social interaction was initially reduced, but she currently attends daycare with improved interaction with peers. Expressive language is limited to one word, but she is able to point at objects. Standardized neurodevelopmental tests (Griffiths III) showed moderate intellectual disability (GQ 74). As soon as she began to grow, it was noted a laryngospasm due

Figure 3 Neonatal MRI Findings



Midsagittal T1-weighted and T2-weighted (A, E), coronal T2-weighted (B, C, F, G), and axial T2-weighted and T1-wighted (D, H); T2-weighted sections from 4 days and 9 months of age postnatal study, respectively, confirming previous findings as regard to corpus callosum (arrows) and ventricular dysmorphism and proportionally more evident vermian (arrowheads) and pontine (asterisks) hypoplasia. Gross appearance of the medulla oblongata and an occipital median skull defect at the level of tentorium insertion (empty arrows), with a T1-hyperintense linear structure that runned deep down to the torcular region, as an intracranial lipoma, are evident too. Moderate enlargement of frontal periencephalic CSF spaces was also noted in the second examination.

to food aspiration. The baby with persistent oropharyngeal dysphagia was fed through a nasogastric tube and percutaneous endoscopic gastrostomy at 3 month Oral and swallowing skills are improving, but most food intake is through PEG. At 20 months, the baby's weight and length parameters were below normal, and she started pneumologic follow-up because of pulmonary dysventilation and food aspiration consequences. Several skeletal abnormalities were described, including bilateral rib dysmorphism with partial fusion defects (I and II ribs on the left and VIII and IX ribs on the right), overlapping toes, partial fusion of C2-C3 metameres, and butterfly vertebra at C4. The MR imaging follow-up at 8 months confirmed cranial findings (Figure 3) and excluded occult dysraphism.

Discussion

The mechanisms by which *HUWE1* gene regulates neuronal function and development are still largely unclear, but a growing body of literature described disease-causing variants in this gene, especially among intellectual disability affected patients.[2,3] We first report extensive description of prenatal and postnatal neuroradiologic phenotype of a female patient carrying a *HUWE1* likely pathogenic variant and discuss its function. In literature, there is no article on genotype-phenotype correlation regarding this *HUWE1* variant. The role of *HUWE1* in brain and cerebellar development has been reported[3,10-12] with correlations to both neural tube defects and medulloblastoma development. The largest case series of patients was described by Moortgat et al.[13]

In addition to intellectual disability, minor face anomalies (long face, a short nose with a broad nasal tip, deep set eyes with epicanthic folds, blepharophimosis, thin upper lip, and full lower lip), and minor SNC anomalies, are described global hypotonia, behavioral disorder (autism spectrum disorder; stereotypies, hyperactivity), skeletal anomalies (short stature, small hands and feet, craniosynostosis, scoliosis, contractures of knees), postnatal microcephaly, and seizure. Less frequently are reported hearing loss, sleep disorder, and feeding difficulties (constipation).[13,14]

Among the described HUWE1-related neuroimaging alterations there are undefined focal cortical malformations; thin corpus callosum; delayed myelination; ventriculomegaly; microcephaly and macrocephaly; isolated cerebellar atrophy; hippocampi of rounded and medialized shape; splenium thickening of corpus callosum; filum terminalis lipoma; unspecific hypoxic-ischemic damage.

In our case, the cerebellar vermis and pontine involvement was one of the key features of the prenatal and postnatal assessment. *HUWE1* plays a key role in the differentiation of cerebellar granule neuron precursors (CGNPs) and the maturation of Bergmann glia during cerebellar development.[10] *HUWE1* rules the interaction between the Bergmann glial scaffold and granule neurons, which is essential for granule neuron migration and IGL formation. Mutations in *HUWE1* can lead to abnormal cerebellar development, misaligned Purkinje cells, disruption of CGNPs differentiation, and disorganization of cerebellar architecture, potentially causing abnormal cell differentiation and tumorigenic states like medulloblastoma.[12] Transcriptional abnormalities in cerebellar formation may directly or indirectly affect brainstem (pons) development. Although initial studies on "cultured ES cells"[15] and Drosophila have been performed, still nothing is known about how a *HUWE1* mutation may result in pathologic conditions on cerebellar development and function. Our patient's posterior median skull defect and intracranial lipoma indicate the role of X chromosome genes in neural tube defects through p53 signaling process alteration[11]; however, the impact of *HUWE1* missense mutation on neural tube closure at the tissue morphogenesis remains unexplored. The short and thin corpus callosum may be due to altered axon development because *HUWE1* influences axon termination or branching in flies and worms.[3]

In conclusion, this report highlights *HUWE1* possible role in CNS development and may help prenatal diagnostic experts in consider also this entity, especially when dealing with pontine and cerebellar hypoplasia findings.

Study Funding

The authors report no targeted funding.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* March 8, 2024. Accepted in final form May 8, 2024. Submitted and externally peer reviewed. The handling editor was Editor Stefan M. Pulst, MD, Dr med, FAAN.

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Appendix *(continued)*

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