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HUWE1 variants cause dominant X-linked intellectual disability: a clinical study of 21 patients

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Abstract

Whole-gene duplications and missense variants in the *HUWE1* gene (NM_031407.6) have been reported in association with intellectual disability (ID). Increased gene dosage has been observed in males with non-syndromic mild to moderate ID with speech delay. Missense variants reported previously appear to be associated with severe ID in males and mild or no ID in obligate carrier females.

Here, we report the largest cohort of patients with *HUWE1* variants, consisting of 14 females and 7 males, with 15 different missense variants and one splice site variant. Clinical assessment identified common clinical features consisting of moderate to profound ID, delayed or absent speech, short stature with small hands and feet and facial dysmorphism consisting of a broad nasal tip, deep set eyes, epicanthic folds, short palpebral fissures, and a short philtrum.

We describe for the first time that females can be severely affected, despite preferential inactivation of the affected X chromosome. Three females with the c.329G>A p.Arg110Gln variant, present with a phenotype of mild ID, specific facial features, scoliosis and craniosynostosis, as reported previously in a single patient. In these females the X inactivation pattern appeared skewed in favour of the affected transcript.

In summary, *HUWE1* missense variants may cause syndromic ID in both males and females.

Introduction

The *HUWE1* gene (HECT, UBA and WWE domain containing 1, E3 ubiquitin protein ligase; MIM 300697), located on Xp11.22, encodes a large protein of 4374 amino acids initially identified in oncogenesis.1 Mouse models have indicated the important role of HUWE1 in the control of neurogenesis in the cerebral cortex via the N-Myc pathway.2–4 Deletion of *Huwe1* in the progenitors of the embryonic mouse brain or only in cerebellar neuron precursors and radial glia, leads to neonatal lethality.3 4

The association of *HUWE1* variants or rearrangements with X-linked intellectual disability (XLID) is now well recognized. In 12 families with mild to moderate non-syndromic XLID, Froyen et al. identified overlapping microduplications at Xp11.22, encompassing *HUWE1* and *HSD17B10,* ranging in size between 0.4 and 1Mb.5 6 With high-resolution mapping of the 12 copy-number gains, they showed that *HUWE1* was the only gene in the minimal duplicated region. Moreover, a 2-fold increased expression of *HUWE1* (but not *HSD17B10*) was seen in cell lines from patients with cognitive impairment.6 In the initial report, the authors also identified three different missense variants in *HUWE1* (c.8942G>A p.(Arg2981His); c.12037C>T p.(Arg4013Trp) and c.12559C>T p.(Arg4187Cys)) in 3 families with non-syndromic moderate to profound XLID.5 7 Carrier females are usually described as normal or less symptomatic, which previous authors attribued to protective skewed inactivation of their affected X chromosome.6 Isrie et al. reported the c.12037C>T (p.(Arg4013Trp)) variant in *HUWE1* in another XLID family in which two affected male patients presented with severe intellectual disability (ID), deep set eyes, down-slanting palpebral fissures, tapering fingers and oedema of the hands and feet.8 Missense variants have since been reported in patients with ID, sometimes in association with autism or

schizophrenia, but clinical information about these patients are limited.9–14 Recently, Friez et al. described a recurrent c.12928G>C (p.Gly4310Arg) missense variant in *HUWE1* in two unrelated XLID-families, formerly described by Juberg and Marsidi and by Brooks.15–17 In a third family, a c.12188G>A (p.Arg4063Gln) variant was identified in two brothers.15 In all three families, affected male patients shared similar clinical features including severe ID, with absence of speech in all but one patient, short stature, microcephaly, contractures, and dysmorphic features such as blepharophimosis, deep-set eyes and a prominent nose. However the contribution of *HUWE1* variants to intellectual disability in female patients is not well understood. The first female patient with a *de novo* c.329G>A (p.Arg110Gln) variant in *HUWE1* had learning difficulties and craniosynostosis.18 She showed unfavourable skewing of her X-inactivation pattern and expressed only the mutant allele in both lymphoblastoid cells and fibroblasts. In addition, two males with craniosynostosis and global developmental delay with *de novo* c.328C>T (p.(Arg110Trp)) have been described, 18–20 adding to the probability that p. Arg110 missense variants cause a specific phenotype.

Here, we describe the clinical and molecular findings in the largest cohort to date of 14 female and 7 male patients with *HUWE1* variants, and review the current literature on *HUWE1* aberrations.

Patients and Methods

Patients

The 21 patients were assessed clinically by at least one of the authors. Written informed consent was obtained for genetic studies and publication of all photographs. All patients were referred for investigation of syndromic/non syndromic developmental delay and/or intellectual disability of unknown cause. Nine patients were ascertained through the Deciphering Developmental (DDD) Study21 (See Table 1 for associated DECIPHER ID numbers).

Four patients have been previously reported (P2, P8, P13 and P14)18 22 23. For each of them, the clinical data has been reassessed. P13 and P14 were clinically described by Verloes et al.23 and included in our cohort because the molecular diagnosis had not yet been identified. In total, we describe 21 individuals, 14 females and 7 males, with an age range of 18 months to 31 years.

Genetic studies

Blood samples from individuals and their parents were collected for trio whole exome sequencing (WES: P3-P8, P9-P15, P17-P19), trio WES with filtering for intellectual disability-genes (ID-panel: P1 and P16) or X-chromosome Exome Sequencing (XES: P20-P21) (Table 1).

The molecular methods and bioinformatic pipeline for WES used in the DDD Study have been previously described21. All variants in the *HUWE1* gene (NM_031407.6) were confirmed by Sanger sequencing. They were interpreted and classified according to the ACMG 2015 Guidelines24. Variants were submitted to the ClinVar database (https://

www.ncbi.nlm.nih.gov/clinvar/). ClinVar accession numbers are listed in Supplementary Table S1.

For the X chromosome inactivation (XCI) assay, we used the polymorphic CAG_n repeat within the human *AR* gene to assess the relative methylation status of both chromosomes after methylation-sensitive restriction enzyme digest to assess the XCI patterns as previously described25. X-inactivation was considered skewed (non random) if the ratio of the two alleles exceeded 80:20 (we refer to 'extreme skewing' where the ratio exceeds 90:10)26. XCI profiles were performed in peripheral blood leucocytes from individuals P1, P3-P5, P7, P9-P10, P15-19, and in the mother and grandmother of P20 and P21 (Table 1 and Figure 1). The analysis was also performed on a buccal swab sample of P1, P11, P15- P17, and in cultured fibroblasts in P1, P4, P5, P7, P15 and P16.

RNA isolation and sequencing of cDNA

Total RNA was isolated from fibroblasts from patient P1, P4, P5, P7, P15, P16 and from blood samples obtained from P1, P4, P11, P17 and controls, by using the RNeasy Mini Kit (QIAGEN) for fibroblasts and PAXgene Blood RNA Kit (QIAGEN) for blood samples, following the manufacturer's protocol. cDNA was synthesized following the manufacturer's protocols. The different variants were amplified by PCR from the patient's cDNA (amplified from total RNA by RT-PCR) from fibroblasts and blood by primers spanning the different variants. The primer sequences can be obtained by request. Polymerase chain reaction (PCR) amplification and the sequencing reactions were performed by standard methods. RNA studies of patient P2 were previously described.18

Results

HUWE1 variants

We report 21 patients with 16 different *HUWE1* variants (Table 1, Figure 1). In 17 cases the *HUWE1* variants were *de novo*. In two unrelated families (Figure 1), two male patients both had a maternally inherited mutation. With the exception of a *de novo* splice-site variant identified in P5, all the missense variants predict substitutions that affect highly conserved amino acids. None of the variants were reported in control databases (Exome Variant Server, 1000Genomes, HapMap, Exome Aggregation Consortium), while the c.9208C>T p.(Arg3070Cys) variant was reported in the ClinVar-database (P11). This variant is recurrent, found in P11 and P12. The recurrent c.329G>A p.Arg110Gln variant found in P1 and P3 was identified in a previously reported patient18 included in our cohort (P2) with updated and detailed clinical findings. The 16 different variants are located in five out of six different functional domains of the protein (Figure 2), but with an overrepresentation (7/16) in the catalytic HECT-domain.

XCI and cDNA results

The majority of female patients tested for XCI (13/14) had a skewed X-inactivation ratio (>80%) except in P9 (Table 1, Figure 3). P7 was non-informative, but cDNA sequencing indicated extreme skewing, as no mutant allele was expressed in RNA. Unaffected heterozygous carrier females (mothers, aunts and grandmother of P20 and P21) also

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presented with skewed XCI (Figure 1). XCI was tested in DNA from different tissues (blood, buccal swab and cultured fibroblasts) in P1, P4, P5, P15, P16 and P17 (see Table1 and Figure 3). In P1, the XCI-pattern was consistent. In P4 and P5 the pattern differed. P4 had random inactivation (60/40) in blood but skewed in fibroblasts (84/16). In contrast, P5 had an extremely skewed pattern in blood (95/5), with a random pattern in the fibroblasts (72/28). In P15, the XCI-pattern was concordant between blood and buccal swab (100/0) while it was random (60/40) in the cultured fibroblasts. In P16, the pattern differed, from 100/0 in blood, 92/8 in cultured fibroblasts, and 69/31 (random) from buccal swab. In P17, XCI was skewed (91/9) in the blood and random (74/26) in buccal swab.

To determine whether the wild type or the mutated allele was preferentially expressed, we performed cDNA sequencing (see Figure 3). In P1, the mutated allele was preferentially expressed in the blood and exclusively expressed in the fibroblasts. A reversed pattern was demonstrated in P4 with preferential expression of the mutated allele in fibroblasts versus wild type in blood. In P5, the splice site variant c.567+1G>C occurred in a splice-donor site in intron 8. cDNA analysis from fibroblasts confirmed that the variant resulted in an abnormal but expressed RNA-product with skipping of exon 8 (NG_016261.2), leading to an in-frame deletion of 21 amino acids. cDNA sequencing could not determine which allele was preferentially inactivated in fibroblasts (XCI 72/28 in fibroblasts). In P7, P15 and P16, the wild type allele was preferentially or exclusively expressed in the fibroblasts, as was seen in P11 and P17 in the blood. In P11, the XCI was only analysed in buccal swab, where it showed a random pattern (66/34), but RNA analysis from blood showed exclusive expression of the wild type allele, indicating an extremely skewed XCI in blood.

Clinical features

All individuals presented with ID (Table 1). 16/19 had severe to profound ID whereas three were mildly affected (P1, P2, P9) and P3 is too young to be assessed (P13 died at 9 months from status epilepticus). The majority had global developmental delay (20/21). Hypotonia was present in 14/20. The mean age of walking was 2 ½ years while three patients never achieved autonomous walking (P4, P5, P16) and one patient lost the use of the walk at 7 years old due to contractures of knees (P15). 17/19 had speech delay, with absence of speech (or less than 5 words) in 13/19 patients. Microcephaly (-2.5 to -7 SD) occurred in 11/21, and was often postnatal in onset. Seizures were reported in 7/18 patients with onset between 9 months and 13 years.

Brain MRI was normal in 9 patients. Thin corpus callosum was noted in 2 and enlarged cerebral ventricles in 4. Autistic features were reported in 7 patients as well as hand stereotypies (8/17).

Low birth weight was noted in 4 patients and postnatal short stature (-2.5 to -6 SD) reported in 15/21. Skeletal anomalies (Figure 4b) included small hands and feet (12/21), overlapping toes (9/21), craniosynostosis (2/21), scoliosis (3/21) and contractures of knees (5/17).

Less common features included hyperpilosity (4/21), hearing loss (4/18), sleep disorder (5/21), hyperactivity (4/16), cryptorchidism (3/8) and hypertonia of lower limbs (2/21). Constipation occurred in 4 patients and feeding difficulties in 2.

Dysmorphic facial features (Figure 4a) frequently reported included a long face (11/21), a broad nasal tip (19/21), a short philtrum (10/21) with thin upper lip (16/21) and full lower lip (8/21), low set ears (7/19) or posteriorly rotated ears (8/19). The eye anomalies were most highly characteristic with deep set eyes (15/21), epicanthic folds (14/21) and blepharophimosis (12/21). Strabismus was reported in 13/20, hypermetropia +/- astigmatism in 9/18 and retinopathy in 3/13.

Discussion

HUWE1 encodes an E3 ubiquitin ligase ubiquitously expressed and important in neuronal development.2–4 In human EBV-cell lines, there is no difference between *HUWE1* expression in males and females, suggesting the gene does not escape X-inactivation.6 Female embryonic stem cell studies confirm that *HUWE1* is subject to XCI but at a later stage of differentiation.27 *HUWE1* is a dosage-sensitive gene initially identified in non-syndromic XLID families with microduplication Xp11.22 encompassing *HUWE1*.5,6 In a *Drosophila melanogaster* model, a 2-fold overexpression of *Huwe1* was not associated with structural brain anomalies, however abnormal axon branching of dorsal cluster neurons through the Wnt/β-catenin pathway was demonstrated.28

We report here on the largest cohort of individuals with *HUWE1* variants, consisting of 21 patients with 16 different variants. All but one were missense, a pattern which concurs with all previously reported patients.5 8–12 15 18–20 22 29

HUWE1 is a large gene, but almost devoid of truncating variants in the ExAC database. The calculated pLI (probability of Loss-of-Function intolerance) is 1.0, and genes with pLI ≥0.9 are considered extremely LoF intolerant. Missense variants in *HUWE1* are extremely rare, compared to the expected, and the ExAC database has calculated a z-score of 8.75, which is highly significant. This is also reflected in our series, where all affected sequences are highly conserved on both nucleotide and amino acid level, along with CADD-scores above 20 (Supplementary Table S1).

The physiopathological mechanisms responsible for alteration of HUWE1 function are still uncertain. Both duplications and missense variants have been associated with intellectual disability suggesting that increased (GOF) or decreased (LOF) function of HUWE1 could cause the disease. Whole *HUWE1* gene deletions and truncating mutations have not been reported in males so far. Furthermore the high rate of perinatal lethality in *Huwe1* knockout mice would suggest that complete loss of function is likely to be incompatible with embryonic development. 2 4 6 Moreover, functional studies on lymphoblastoid cell lines derived from affected male patients have already suggested that missense variants in *HUWE1* could affect its function through different mechanisms. 15 Indeed, for a p.Gly4310Arg affected male patient, the authors found reduced protein levels of HUWE1 compared to healthy relative's cells as well as accumulation of Mcl1 and p53, downstream targets of HUWE1. But on the other hand, for an affected patient with p.Arg4063Gln, increased HUWE1 protein levels were observed with consequently reduced levels of p53 substrates. For others, missense variants have led to increased auto-ubiquitination of HUWE1 in vitro. 30 In our cohort, the consequences of the missense and the splice site

variants are not yet understood. We hypothesize that the p.Arg100Gln variant responsible for a specific phenotype could result from a different molecular mechanism compared to the other missense variants reported. Additional functional studies in our series will be necessary to further understand the impact of these variants. These studies would need to compare HUWE1 and target proteins levels using available patients' fibroblasts, and to study HUWE1 protein stability with overexpression of mutants in cellular models.

Prior to this work, 26 male patients from 9 unrelated families and 11 sporadic male and female individuals with missense *HUWE1* variants have been reported in the literature5 8–12 15 18 19 22 29. We have excluded from our discussion 6 sporadic patients whose descriptions lack accurate phenotypic information.9–12 29 All 31 published males have moderate to profound ID, which is a consistent feature that we found in our study, confirming that *HUWE1* variants are associated with significant ID. Absent or limited speech is reported in 75 % of published patients and in 89 % of our cohort. Postnatal short stature occurred in 70% of patients in the literature, with the same rate in our series (71%). We highlight recurrent facial dysmorphism in our patients and those previously reported, consisting of a long face, a short nose with a broad nasal tip, deep set eyes with epicanthic folds, blepharophimosis, thin upper lip and full lower lip. However, the dysmorphic features do not present as a specifically recognisable gestalt. Skeletal anomalies consist of small hands and feet (57%), overlapping toes (42%) and contractures of the knees and other large joints. This last characteristic was previously reported.5 7 15–17 Ophthalmologic anomalies must be screened for, in particular strabismus (65% of all patients), hypermetropia and/or astigmatism (50%).

Of note five patients have variants affecting the same residue p.Arg110 of HUWE1. Three were female patients from this cohort with the c.329G>A p.Arg110Gln substitution (P1-P3), and the other two were male patients with c.328C>T p.(Arg110Trp) variant who have previously been reported in the literature.18–20 Both male p.(Arg110Trp) patients presented with craniosynostosis, ID and Chiari malformation. One had the additional features of scoliosis, shortening of digits, small toes with 4-5 syndactyly and facial dysmorphism (flat midface, downslanting palpebral fissures and low set ears) which is different to the dysmorphism we have observed in our cohort.18 20 Of the three female patients with p.Arg110Gln, two have craniosynostosis (multilacunar in P2, lacunar skull defects and unilateral coronal and metopic craniosynostosis in P3). P1 and P2 (p.Arg110Gln) have similar facial dysmorphism with a high forehead, down-slanting palpebral fissures, prominent eyes, flat midface, small nose with hypoplastic nares, nasal and high-pitched voice, and oligodontia. They have short distal phalanges with small nails. Short stature and scoliosis is seen in P1. P1 and P2 have mild ID, while P3 is too young to assess but showing global developmental delay. P4 has a severe neurologic phenotype while the facial phenotype is not typical for the rest of the cohort, but might resemble P1-P3. She also shares Chiari-malformation in common with the p.Arg110Gln-patients. In summary we note that some dysmorphic features are different in patients P1-P4 compared to patients with different variants suggesting a possible specific phenotype.

Robust genotype-phenotype correlations are difficult to establish at this point because of low numbers. In accordance with previously reported cases, the 16 different variants in our

cohort are located in five out of six different functional domains of the protein (Figure 2). However their location does not suggest an obvious explanation for the phenotypic variability in the patients. We noted an overrepresentation of variants (7/16) in the catalytic HECT-domain but could not detect a specific phenotype in these patients. There was no significant difference between the female and male groups either overall or at specific domains.

We also describe two unrelated females with a *de novo* c.9208C>T p.(Arg3070Cys) variant (P10 and P11). P11 was previously reported in the ClinVar-database, but without clinical information. Whilst both females are phenotypically similar to the wider cohort, P10 had severe ID and absence of speech, whereas P11 had moderate ID with late-normal motor milestones. In the literature, we found four recurrent variants (c.8942G>A p.(Arg2981His), c.12037C>T p.(Arg4013Trp), c.12559C>T p.(Arg4187Cys) and c.12928G>C p.Gly4310Arg in *HUWE1).*5 8 15 No obvious genotype-phenotype correlation could be established apart from the recurrent p.Gly4310Arg variant reported in two families who have previously been described as Juberg-Marsidi syndrome and Brooks syndrome respectively.16 17 These patients share in common severe syndromic ID with absence of speech, short stature, contractures, blepharophimosis, epicanthus and deep set eyes, a thin upper lip, cupped ears and a bulbous nose, the same dysmorphism as described in our patients.

We report for the first time a severe phenotype in females with *HUWE1* variants. Previously reported carrier females were described as normal or mildly symptomatic, presumed to be due to the protective effect of skewed X inactivation of the mutated X chromosome. However, in our series, severely affected female patients also had an extremely skewed X-inactivation pattern, and cDNA sequencing revealed almost exclusive expression of the normal allele (P7, P11, P15, P16 and P17). As the pattern of skewing observed in blood may not be representative of all tissues, differential X inactivation patterns in different tissues may explain the different phenotypes. The buccal swab sample (P1, P16 and P17) and cultured fibroblasts of P1 demonstrate this. Patients P1, P2 and P3 with c.329G>A p.Arg110Gln variants all showed a specific phenotype with mild ID plus facial dysmorphism, skeletal anomalies and craniosynostosis, and all had completely skewed inactivation of their X chromosome with preferential inactivation of the wild type X chromosome. This phenomenon has been widely described with X-linked variants,26 31 but the cause of such apparently disadvantageous skewing is not clear. We postulate that this specific *HUWE1* variant could be responsible for this specific phenotype. We confirmed by cDNA analysis that P1 preferentially expressed the mutant transcript in both leucocytes and fibroblasts. Similar findings were reported for P2 in Taylor et al.13 P4 with c.344C>T p.(Ser115Phe) variant presented an atypical phenotype, and the X-inactivation pattern in fibroblast from her was comparable to the pattern seen in the p.Arg110Gln-patients, with unfavourable skewing.

In conclusion, patients with variants in *HUWE1* typically present with moderate to profound ID, short stature, severe speech difficulties and nonspecific but recurrent dysmorphic facial features such as deep-set eyes and a broad nasal tip. In males, the majority of variants are *de novo*, a minority are maternally inherited from healthy female carriers demonstrating an extremely skewed pattern of x inactivation in favour of the normal X. We have identified

HUWE1 variants in female patients with a severe syndromic ID phenotype. Clearly affected females have *de novo* variants with skewed XCI. The c.329G>A p.Arg110Gln variants are associated with a specific phenotype including craniosynostosis, and with an unexpected XCI-pattern favouring the mutated allele. It is now important to identify additional individuals with *HUWE1* variants to confirm the phenotypes related to this gene, and to clarify genotype-phenotype correlations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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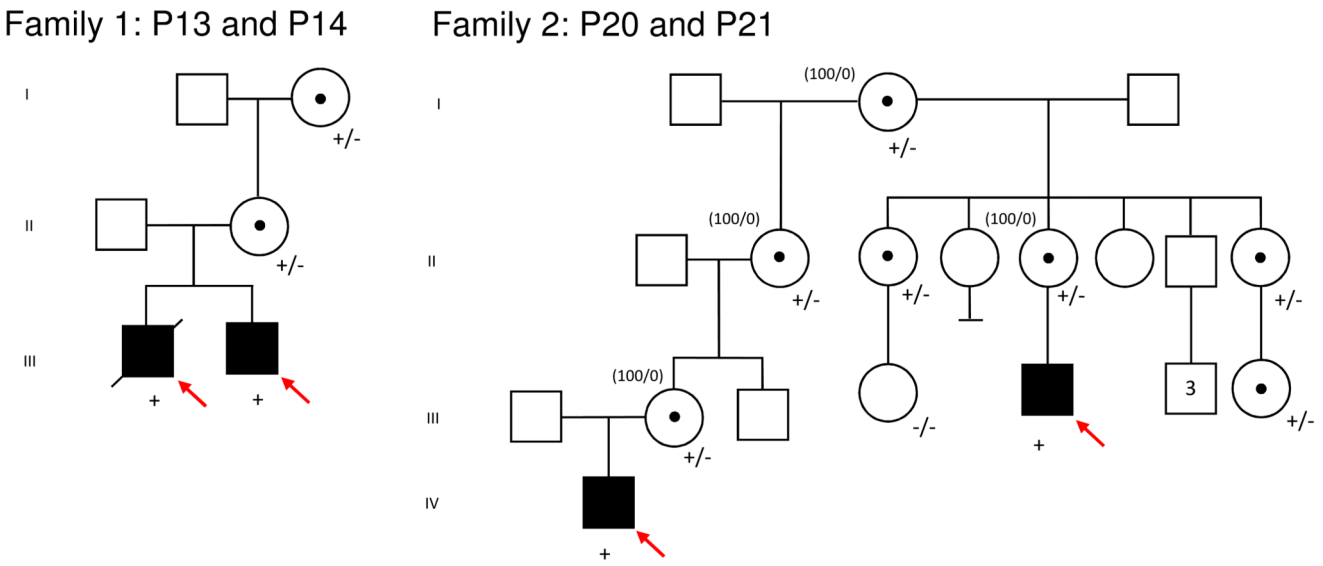

Figure 1.

Pedigrees of families with maternally inherited *HUWE1* variants. Red arrow indicates the proband; +, hemizygous status; +/-, heterozygous status, -/-, wt. Numbers in brackets indicate the XCI pattern in female carriers.

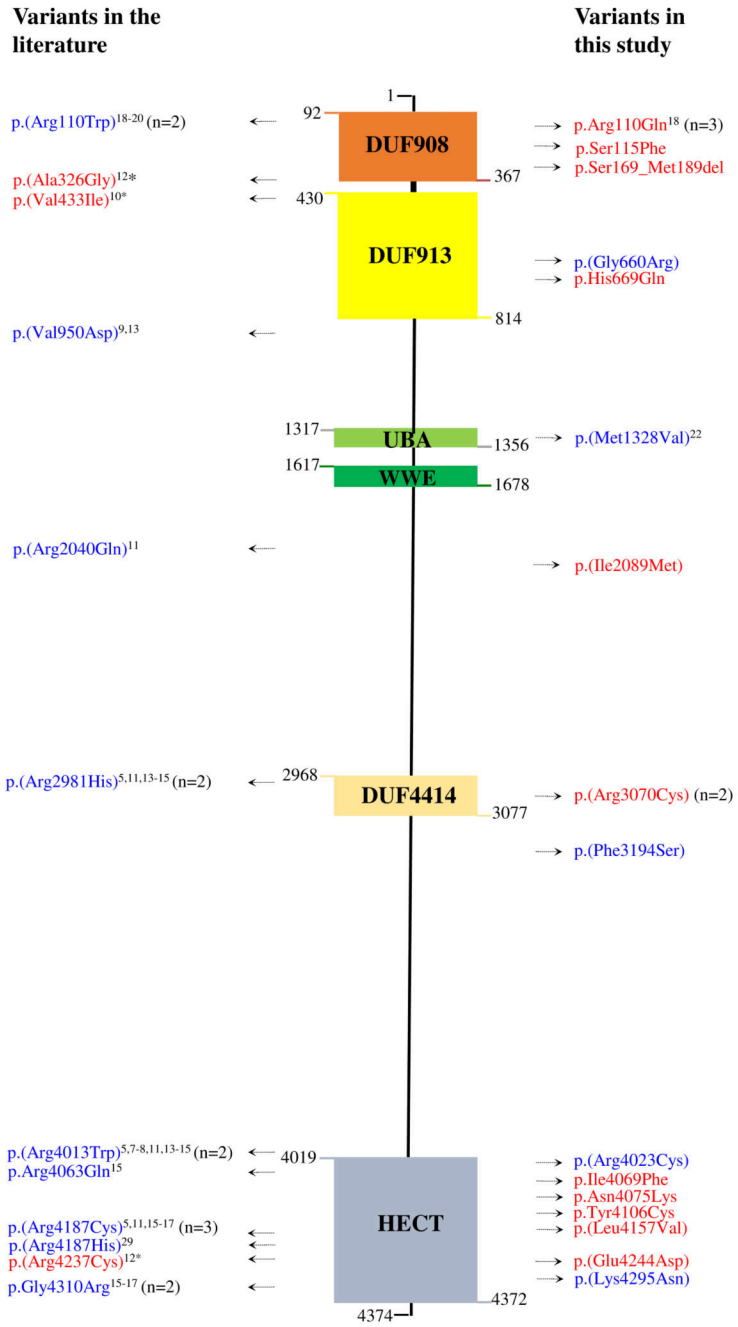

Figure 2.

Schematic representation of HUWE1 protein with identified variants. (Refseq: NM_031407.6, NCBI Protein Reference Sequence: NP_113584.3).

The amino acid-borders of the described HUWE1 domains are noted. Previously published variants are shown on the left and variants in the present cohort on the right. Only rare non-synonymous and splice site variants are presented.

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Male patients are in blue and female in red. Number in parenthesis indicates recurrent variants describing number of families reported for the variant. *Females with schizophrenia as indication for WES with *de novo HUWE1* missense changes.
Abbreviations: DUF = domain of unknown function; UBA = ubiquitin-associated; WWE = tryptophan tryptophan glutamate; HECT = Homologous to the E6-AP Carboxyl Terminus.

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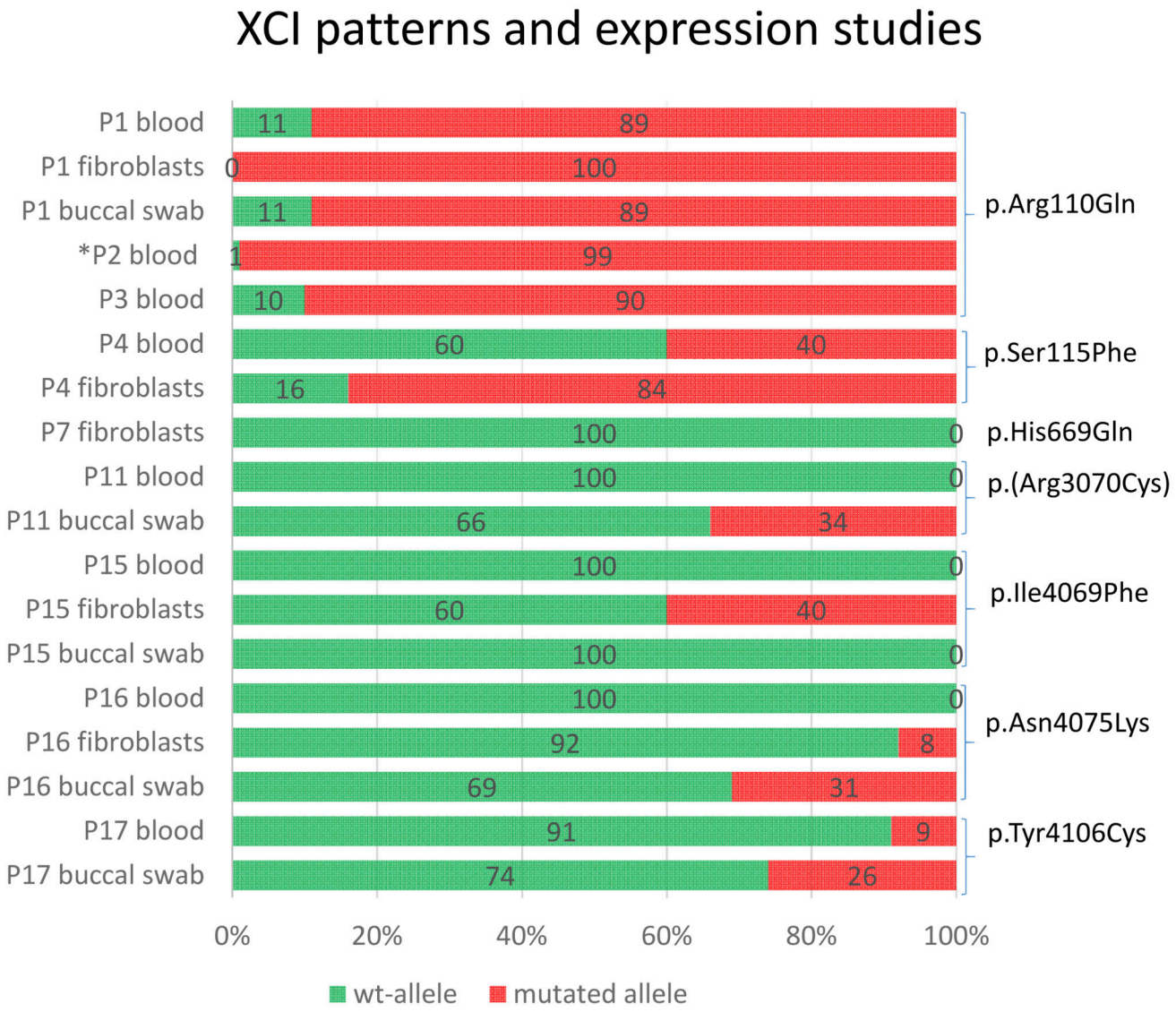

Figure 3.

X-inactivation patterns in female patients in varying tissues and associated RNA expression analysis. cDNA-sequencing was used to determine which allele was preferentially expressed; the green colour indicated the expression pattern of the wild type allele and the red, the expression pattern of the mutated allele. The numbers superimposed on bars represent XCI in the specified tissue. *P2 analysed by Taylor et al. In P7, XCI was non-informative due to homozygous AR-alleles, but wt-allele exclusively expressed in blood. In P11, cDNA analysis was performed in one tissue demonstrating preferential expression of the wild type allele. This was assumed to be true for both tissues.

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Figure 4.

Facial and limb features of individuals with *HUWE1* variants, frontal and lateral views. **a)** Facial features of all but two patients in the cohort. Patient numbers correspond to those in the text and tables. Detailed description of facial phenotype can be found in Table 1. Note the particular phenotype in P1 to P3 (carrying the same *de novo* c.329G>A p.Arg110Gln variant) consisting of a flat face, prominent eyes and a small nose, compared to other patients (P4-P20) who present with deep-set eyes, epicanthic folds, blepharophimosis, broad nasal tip and thin upper lip. The facial shape seems to evolve with time, from round face

with full cheeks to long face, as seen in P6, P19 and P20. **b)** Skeletal features include small hands, short distal phalanges and short nails in P1-P3, clinodactyly of 5th fingers, small nails, short metatarsals and 3rd-5th toes in P1 and P2, 2-3 syndactyly in P1 and P3. Tapering of fingers and puffy hands are shown in P8, and overlapping toes in P16 and P17.

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Eur J Hum Genet. Author manuscript; available in PMC 2018 July 01.

Table 1

Clinical and molecular features from the 21 patients with HUWE1 variants*

Patient	P1	P2	P3	P4 (DDD 325588)	P5	P6 (DDD 271657)	P7 (DDD 261673)	P8	P9 (DDD 263322)	P10 (DDD 274065)	P11	P12 (DDD 264956)	P13	P14	P15 (DDD 273584)	P16	P17 (DDD 279843)	P18	P19 (DDD 259868)	P20	P21	Total cases n=21	percentage (%)
Age at genetic diagnosis (y)	14	4	1.5	18	5	7	6	10	7	29	6	5	NA	13	15	3	3	11	11	11	31		
Sex	F	F	F	F	F	M	F	M	F	F	F	M	M	M	F	F	F	F	F	M	M	14F/7M	
HUWE1 variant	c.329G>A p.Arg110Gln	c.329G>A p.Arg110Gln	c.329G>A p.Arg110Gln	c.344C>T p.(Ser115Phe)	c.567+1G>C p.Ser169_Met189del	c.1978G>A p.(Gly660Arg)	c.2007T>G p.His669Gln	c.3982A>G p.(Met1328Val)	c.6267T>G p.(Ile2089Met)	c.9208C>T p.(Arg3070Cys)	c.9208C>T p.(Arg3070Cys)	c.9581T>C p.(Phe3194Ser)	c.12067C>T p.(Arg4023Cys)		c.12205A>T p.Ile4069Phe	c.12225C>G p.Asn4075Lys	c.12317A>G p.Tyr4106Cys	c.12469C>G p.(Leu4157Val)	c.12732G>C p.(Glu4244Asp)	c.12885G>C p.(Lys4295Asn)			
Inheritance	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	Mat/grandMat		De novo	De novo	De novo	De novo	De novo	Mat	Mat	4Mat/17 de novo	
Test method	ID-panel	WGS	WES	WES	WES	WES	WES	WES	WES	WES	WES	WES	WES		WES	ID-panel	WES	WES	WES	XES			
XCI-in pattern	89/11 (B; BS), 100/0 (Fb)	99/1 (B)	90/10 (B)	60/40 (B), 84/16 (Fb)	95/5 (B), 72/28 (Fb)		NINE but cDNA seq indicates extreme skewing		67/33 (B)	81/19 (B)	66/34 (BS), cDNA seq from blood indicates extremely skewing				100/0 (B; BS), 60/40 (Fb)	100/0 (B), 69/31 (BS), 92/8 (Fb)	91/9 (B), 74/26 (BS)	100/0 (B)	90/10 (B)				
Growth findings																							
Normal birth parametres	+	+	+	-	+	+	+	+	+	+	+	+	-	- (microcephaly)	+	-	+		-	+	+	15/20	75
Height <3rd centile (SD)	+ (-3)	-	-	+ (-2,5)	+ (-6)	-	+ (-3,5)	+ (-2,5)	-	+ (-4)	+	+ (-4)	+ (-2,7)	+ (-6)	+	+	+ (-4)	-	-	+ (-2,5)	+ (-4,5)	15/21	71
Weight <3rd centile (SD)	+ (-3,8)	- (-1,9)	-	+	+ (-6)	-	-	-	-	-	+	+ (-2,2)	+ (-2,3)	+ (-4)	-	+	+ (-3,5)	-	-	+	+ (-4)	11/21	52
Microcephaly (SD)	-	-	+ (-2,3)	+ (-2,5)	+ (-4)	-	+ (-4)	-	-	+ (-4)	-	-	+ (-3,1)	+ (-7)	+	-	+ (-6)	+ (-2,5)	+ (-4)	-	-	11/21	52
Facies																							
Hypotelorism	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	+	+	+	-	-	6/21	28
Hypertelorism	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	7/21	33
Small nose	+	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	-	-'	-	-	-	8/21	38
Broad nasal tip	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	19/21	90
Short philtrum	+	-	-	-	-	-	+	+	-	+	-	-	+	+	+	+	-	+	-	+	-	10/21	47
Full lower lip	-	-	-	+	-	-	+	+	-	-	-	-	+	+	+	-	-	+	+	-	-	8/21	38
Thin upper lip	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	+	+	+	+	+	16/21	76
Long face	-	-	+	+	-	-	-	+	+	+	-	-	-	+	+	-	-	+	+	+	+	11/21	52
High forehead	+	+	+	+	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	13/21	61
Other	flat midface, small nasal tip	flat midface, small nasal tip, arched palate	short neck		short neck							anterior flammus naevus	cleft palate, retrognathia, thick columella, narrow mouth	cleft palate		preauricular pit							
Eyes																							
Deep set eyes	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	15/21	71
Epicanthic folds	-	+ (telecanthus)	-	-	+	+	+	+	+	-	-	+ (telecanthus)	+	+	+	-	+	-	+	+	+	14/21	66
Blepharophimosis/ short PF	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	12/21	57
Strabismus	-	+	+	-	+	+	+	+	-	+	-	+		-	-	+	+	+	-	+	+	13/20	65
Retinopathy	-	-	-	-	+ (early stage)			+	-		-			-	-	+	-	-	-	-	-	3/16	18
Refraction error	-	-	-	-	hypermetropia	hypermetropia			-		astigmatism	hypermetropia (severe)		-	-	hypermetropia (severe)	hypermetropia (severe)	astigmatism	-	hypermetropia, astigmatism	hypermetropia, astigmatism	9/18	50

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Patient	P1	P2	P3	P4 (DDD 325588)	P5	P6 (DDD 271657)	P7 (DDD 261673)	P8	P9 (DDD 263322)	P10 (DDD 274065)	P11	P12 (DDD 264956)	P13	P14	P15 (DDD 273584)	P16	P17 (DDD 279843)	P18	P19 (DDD 259868)	P20	P21	Total cases n=21	percentage (%)
Other	slight downslanting PF	slight downslanting PF, prominent eyes		upslanting PF	end gaze nystagmus			downslanting PF, atrophic optic nerve, nystagmus, photophobia	upslanting PF								mild nystagmus						
Ears																							
Low set	+	-	-	+	-	+	-	-	+		-	+		-	-	-	+	+	-	-	-	**7/19**	**36**
Posterior rotated	+	-	-	+		+	-	+	+		-	+	+	-	-	-	+	-	-	-	-	**8/19**	**42**
Hearing loss	-	+	-	-		-		-	-	-	-	SNHL	+, SNHL		-	+	-	-	-	-	-	**4/18**	**22**
Hands and Feet								-															
Brachydactyly or small hands	+ left simian crease	+	-	-	+	+	+	-	-	-	-	+	adducted thumbs, deep palmar creases	short and adducted thumbs	+ tapered fingers	+	-	-	-	+	+ (dermatoglyphic anomalies)	**12/21**	**57**
Small nails	+	+	-	-	-	-		-	-		-			-	-	-	-	-	-	+	+	**4/17**	**23**
Skeletal anomalies																							
Overlapping toes	-	+	-	-	+	-	+	-	+	-	-	-	+	+	-	+	+	-	-	+	-	**9/21**	**42**
Flexum contractures	-	-	-	-	-	-	-	+			-	-			+ (progressive)	+	-	-	-	+	+	**5/17**	**29**
Other	scoliosis, short 3rd-4th metatarsus left and 3rd right foot, short distal phalanges	multilacunar craniosynostosis, short 3rd-5th metatarsal, short distal phalanges	craniosynostosis (coronal/ metopic), lacunar defects skull, 2-3 syndactyly of toes	hemangioma in left humerus, osteoporosis		congenital bilateral hip dislocation		scoliosis, pes panus			sandal gap				scoliosis	patella dislocation, congenital bilateral hip dislocation	fingers curve towards midline	-		pes valgus	hyperlordosis		
Neurological																							
Global motor delay	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**20/21**	**95**
Sitting without support (months)	8	8	12			10		13		24	10		-		10	54	12	10	9	12	15		
Walking (years)	1	2	NA	no walk	no walk	2	6	4.2	2	2	1.5	2	NA	-	no walk	no walk	-	3	3.5	1.5	3.5	**16/19 (delayed)**	**84**
Absence of speech (or <5words) or DS	-	-, DS	NA	+	+	+	+	+	-, DS	+	-	+	NA	+	+	+	+	+	-, DS	-, DS	+	**17/19**	**89**
Mild ID	+	+	NA			-			+				NA	-	-	-	-					**3/19**	**16**
Moderate/Severe ID	-	-	NA	+	+	+	+	+	-	+	moderate	+	NA	+ (profound)	+	+ (profound)	+ (profound)	+	+	+	+	**16/19**	**84**
Hypotonia	-	+	-	+	+	-	+	+	-	-	+	+	+		+	+	-	+	+	+	+	**14/20**	**70**
Seizures (age of onset in years)	-	+ (9)	-	+	-			+ (9)	-	-	-		+ (<1)	+ (1)	-	-	-	+	-	+ (13)	-	**7/18**	**38**
Brain MRI anomalies	-	Arnold Chiari malformation; traumatic brain damage from surgery removing multiple bone spiculae		Arnold-Chiari malformation	thin CC; delayed myelination	-		thin CC, decreased white matter, EV	-	-		-	mild EV		-	-	-	-		mild EV	mild EV	**7/16**	**43**
Other	Mild digitate cranial impressions on X-ray, platybasia on CT			poor sleep, hypertonia					poor sleep						EEG normal	EEG normal		poor sleep	poor sleep	lower limbs hypoertonia	poor sleep		
Behavioral findings																							
Hyperactivity	-	-	-	-		-		+		+	+	-			-	-	-	+	-	-	-	**4/16**	**25**
Stereotypies	-	-	-	+	+	-		+	-	+		-			-	-	+	+	-	-	+	**8/17**	**47**
Autistic features	-	-	NA	+	-	+	+				+	+						+	+		-		
Additional features																							
	oligodontia, nasal speech, severe feeding	oligodontia., unusual teeth with notches and the				hydronephrosis		hyperpilosity (elbows/back), constipation,				cryptorchidism, umbilical hernia, diastasis	hypospadias	shawl scrotum	OCT deficiency	asymmetry, anterior placed anus,	hyperpilosity (elbows/back), crowded teeth,	constipation		cryptorchidism, constipation, hyperpilosity	cryptorchidism, hyperpilosity (elbows/back),		

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Patient	P1	P2	P3	P4 (DDD 325588)	P5	P6 (DDD 271657)	P7 (DDD 261673)	P8	P9 (DDD 263322)	P10 (DDD 274065)	P11	P12 (DDD 264956)	P13	P14	P15 (DDD 273584)	P16	P17 (DDD 279843)	P18	P19 (DDD 259868)	P20	P21	Total cases n=21	percentage (%)
	difficulties (nasogastric tube during 7 months)	edges of incisors curled backwards, nasal speech						small joints hyperlaxity, shawl scrotum, transitory infantile neutropenia				recti, constipation				constipation first 3 years	prominent clitoris and labia, high pain threshold			(elbows/back), shawl scrotum, infantile feedings difficulties; RGO; transitory and isolated neonatal hypercalcemia	buccal lingual dyspraxia, equilibrated translocation (16;17) (p13,2-q11,2) paternally inherited		

Patients are ordered by mutation from the N' of the protein. Blank indicated not available, not applicable or not measured; RefSeq HUWE1: NM_011407.6. According to HGVS recommandations, variant description at protein level is given without brackets when RNA studies were performed, and between brackets when it was not.

Abbreviations: B, blood; BS, buccal swab; CC, corpus callosum; DS, delayed speech; EV, enlarged ventricles; F, female; Fb, fibroblasts; ID-panel, exome sequencing with filtering for intellectual disability-genes; M, male; Mat, maternal; NA, not available; NINF, not informative; PF, palpebral fissures; SNHL, sensorineural hearing loss; WES, whole exome sequencing; WGS, whole genome sequencing; XES, X-exome sequencing; XCI, X chromosome inactivation;