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SHORT COMMUNICATION

Novel microduplications at Xp11.22 including *HUWE1*: clinical and molecular insights into these genomic rearrangements associated with intellectual disability

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Recently, we defined a minimal overlapping region for causal Xp11.22 copy number gains in males with intellectual disability (ID), and identified HECT, UBA and WWE domain-containing protein-1 (*HUWE1)* as the primary dosage-sensitive gene, whose overexpression leads to ID. In the present study, we used this minimal interval to search for *HUWE1* copy number variations by quantitative polymerase chain reaction in a large cohort of Brazilian males with idiopathic ID. We detected two unrelated sporadic individuals with syndromic ID carrying unique overlapping duplications encompassing *HUWE1*. Breakpoint junction analysis showed a simple tandem duplication in the first patient, which has probably arisen by microhomology-mediated break-induced repair mechanism. In the second patient, the rearrangement is complex having an insertion of an intrachromosomal sequence at its junction. This kind of rearrangement has not been reported in Xp11.22 duplications and might have emerged by a replication- or recombination-based mechanism. Furthermore, the presence of infantile seizures in the second family suggests a potential role of increased *KDM5C* expression on epilepsy. Our findings highlight the importance of microduplications at Xp11.22 to ID, even in sporadic cases, and reveal new clinical and molecular insight into *HUWE1* copy number gains.
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Xp11.22 duplications associated with X-linked intellectual disability were identified in 11 families with a 0.2 Mb common minimal overlapping region that only contained the HECT, UBA and WWE domain-containing protein-1 (*HUWE1)* gene for which a two- to fivefold overexpression in blood cells from affected individuals was observed.[1,2] The copy number gain was reported to cause mild-to-moderate ID with minor additional clinical features.[1,2]

Herein, we used the minimal common interval (chrX:53,484,944-53,712,957; UCSC Hg19) seen in the previously described Xp11.22 duplications[2] to search for *HUWE1* copy number variations in a cohort of 906 unrelated males, aged 2–39 years (10 ± 5.8), with mild-to-severe idiopathic ID. These individuals were referred to the Human Genetics Service at the State University of Rio de Janeiro (Brazil) from 1994 to 2013, presenting negative results for Fragile X testing and cytogenetic investigation. The presence of a copy number variation at this location was screened for by quantitative polymerase chain reaction using a primer set located at the 5′ end of *HUWE1* (chrX:53,680,709-53,680,780; Supplementary Table 1) and porcupine homolog (*Drosophila*) gene (chrX:48,368,172-48,379,202) as the normalizer.[3]

We identified two patients (611 and 3272) harboring new unique chromosomal rearrangements at Xp11.22 (Supplementary Figure 1). Clinical and genetic data were deposited in Decipher Consortium database (Patient 291035 for 611 and Patient 291036 for 3272).

Patient 611 (Figure 1a; individual III.5), a 17-year-old Caucasian boy, is the only child of an unrelated young couple. He was born by a breech cesarean delivery after an uneventful pregnancy with a birth weight of 2 600 g and length of 47 cm. He walked at the age of 11 months, but global developmental delay was noticed during the first years. Subsequently, he presented with moderate ID,

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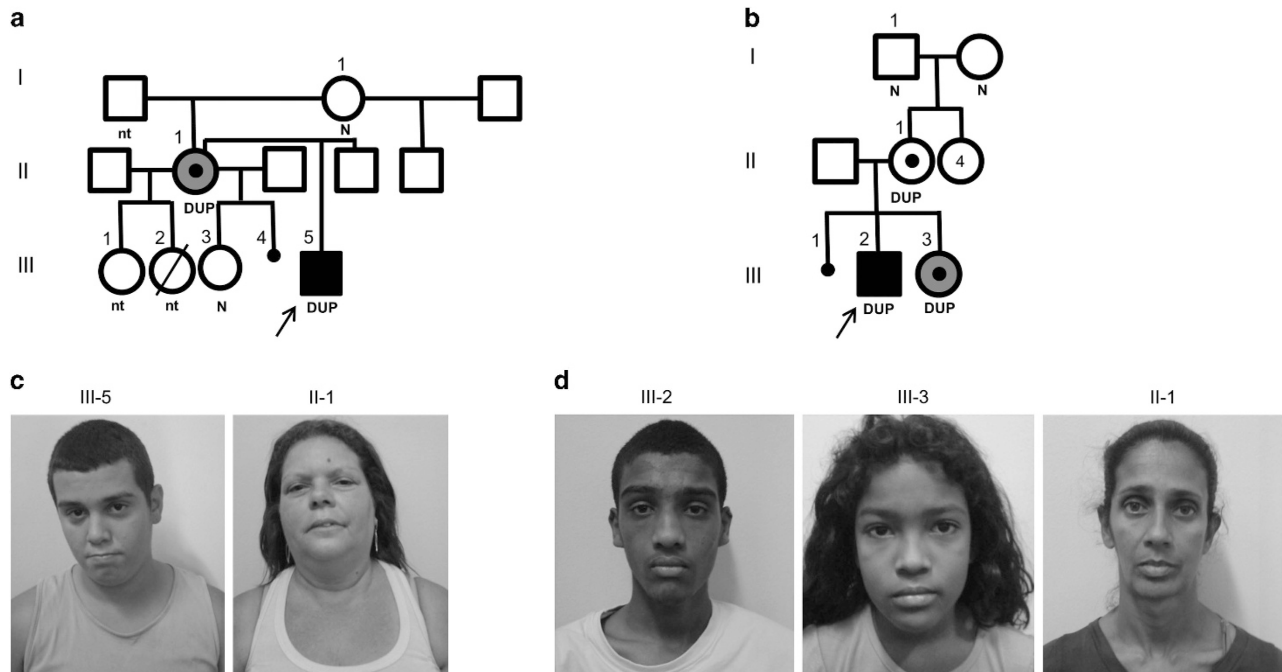

Figure 1 Xp11.22 CNV analysis in families 611 and 3272. Pedigrees of the families 611 (**a**) and 3272 (**b**), respectively, showing the segregation of the Xp11.22 copy number gains (DUP). Solid squares represent boys with ID and circle with a dot represents the carrier females. Open squares represent unaffected males. Patients are shown by arrows. 'N' indicates no duplication. nt, is for 'not available to be tested'. Circles in gray show carrier females with slight intellectual impairment. Abortion was provoked in individual III.4 from family 611 and spontaneous in individual III.1 from family 3272. Pictures of the affected males and heterozygous females with Xp11.22 copy number gain in family 611 (**c**) and 3272 (**d**). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

hyperactivity, language delay, attention problems and aggressive behavior. Currently, at age 17 years, his language skills are poor, he is able to count to 10, write his first name, take a shower and eat independently, although he needs help with the activities of daily living. Physical examination reveals overweight (body mass index = 26.9; $85 < P < 97$), stature at third percentile (162 cm), head circumference at second percentile (53.2 cm), abnormal gait (increased support base with feet externally deviated), brachycephaly, enophtalmia, long face, prominent supraorbital ridges, ocular hypertelorism, large ears with anterior lobes, slight bulbous nose, high arched palate, squared teeth, large thoracic cage, mammillary hypertelorism, inverted nipples, thoracic scoliosis and slight clinodactily of the fifth right finger. No seizures were noted. Magnetic resonance imaging was normal. The patient's mother (Figure 1a; II.1) is a cleaning lady with slight intellectual impairment.

Patient 3272 (Figure 1b; III.2), a non-Caucasian 17-year-old boy, is the first living child of a young non-consanguineous couple. He was born by a breech cesarean delivery after an uneventful pregnancy with a birth weight of 2330 g and 47 cm of length. Developmental milestones include walking at the age of 12 months and speaking of first words at 36 months of age. Besides speech delay, he presented motor incoordination with abnormal gait, moderate ID and hyperactivity. At age 17 years, his weight was 53 kg ($P < 10$), height 176 cm ($P = 50$), with head circumference of 58 cm ($P = 98$) and he walked normally. Clinical findings included flat and 'triangular' facies, malar hypoplasia, prognathism, prominent nasal root, broad nose, enophthalmia, short philtrum, short oral frenula, hypertrophied alveolar ridges, squared small teeth, separated superior central incisors. Normal set ears with malformed auricles, long neck, straight and long thoracic cage, small and sparse nipples, extranumerary nipples, pectus slightly excavatum, cubitus valgus, elbow dysplasia, camptodactily and clinodactily of the fifth fingers, large interphalangeals, calvus feet, nail hypoplasia, short third metatarsus and phimosis. Previous electroencephalogram recordings at 3-year-old showed abnormalities consisting of low-amplitude slow waves mixed with spikes; at 8-year-old, electroencephalogram recordings consisted of high-amplitude spike-wave in posterior regions. Electroencephalogram recordings are consistent with seizures. He has been treated with carbamazepine and risperidone and no more seizures other than those reported at 5 months of age were reported. Brain computed tomography scan revealed no abnormalities.

The 9-year-old sister of patient 3272 (Figure 1b; III.3) attended normal school, but a borderline intellectual functioning and a psychomotor delay was noted. Clinical features included flat and 'triangular' facies, prognathism, prominent nasal root, short philtrum, straight palate, hypertrophied alveolar ridges, squared small teeth, separated superior central incisors and calvus feet. Tonic-clonic seizures were present until 3-year-old. Electroencephalogram recordings (at 7-year-old) showed abnormalities consisting of slow and medium voltage and irregular spike-waves in fronto-temporal regions, predominantly at the left side. The mother (Figure 1b; II.1) is apparently healthy. Magnetic resonance imaging of brain from the patient, sister and mother showed normal results.

Segregation analysis revealed that the mother of patient 611 and the mother and sister of patient 3272 are carriers of the duplications, whereas duplications are absent in the maternal grandmothers. Considering that paternal grandfathers do not present ID and that the duplication was absent on grandfather of patient 3272 (Figure 1c; I.1), it appears that the copy number gain was a *de novo* event in both mothers due to a parental gametogenesis error.

Females presenting *HUWE1* duplications are usually healthy carriers and both skewed and random X-inactivation patterns were reported.[2] X-inactivation status assay at the androgen receptor locus[4] in the borderline mother of patient 611 (Figure 1a; II.1) showed a skewed X-inactivation ratio of 80:20. The carrier sister of patient 3272 (Figure 1b; III.3), who exhibits a more marked phenotype, an extremely skewed ratio of 97:3 was found, whereas the assay was not informative in the healthy carrier mother of this patient (Figure 1b; II.1).

Fine mapping with iterative rounds of quantitative polymerase chain reaction[2] showed that in patient 611, the duplication has 758 Kb (ChrX:53,316,256-54,074,258) spanning seven annotated RefSeq genes (*SMC1A*, *RIBC1*, *HSD17B10*, *HUWE1*, Mir-98, Mir-let7f-2 and *PHF8*) (Figure 2). The duplication in patient 3272 (Figure 1b; III.2) was 905 Kb in size (ChrX:53,228,169-54,133,735) and next to the genes included in the patient 611, also included *KDM5C* and *IQSEC2* distal, and *FAM120C* proximal to this region (Figure 2). Six genes involved in the duplication of 3272 are X-linked ID genes (*KDM5C*, *IQSEC2*, *SMC1A*, *HSD17B10*, *HUWE1* and *PHF8*), from which only lysine (K)-specific demethylase 5C (*KDM5C;* MIM 314690) has a role in epilepsy.[5] Interestingly, patient 3272 and his carrier sister but not patient 611 presented infantile seizures, which could be due to *KDM5C* overexpression (Supplementary Figure 2). Although isolated *KDM5C* duplications have not been reported, the majority of patients with deleterious mutations usually exhibit seizures[6] and *KDM5C* target genes involved in epilepsy have been previously described. [5] Besides, a recent study suggested that *KDM5C* is directly regulated by the Aristaless-related homeobox (*ARX*), a well-established X-linked intellectual disability gene involved in epilepsy.[7] Of note, the medical records of the previously reported Brazilian patient ON1[2] points to the use of anti-epileptical drugs (Haldol and Carbamazepine) at 5-year-old. *KDM5C* overexpression about three- to sixfold was quantified in both 3272 and ON1 patients (Supplementary Figure 2).

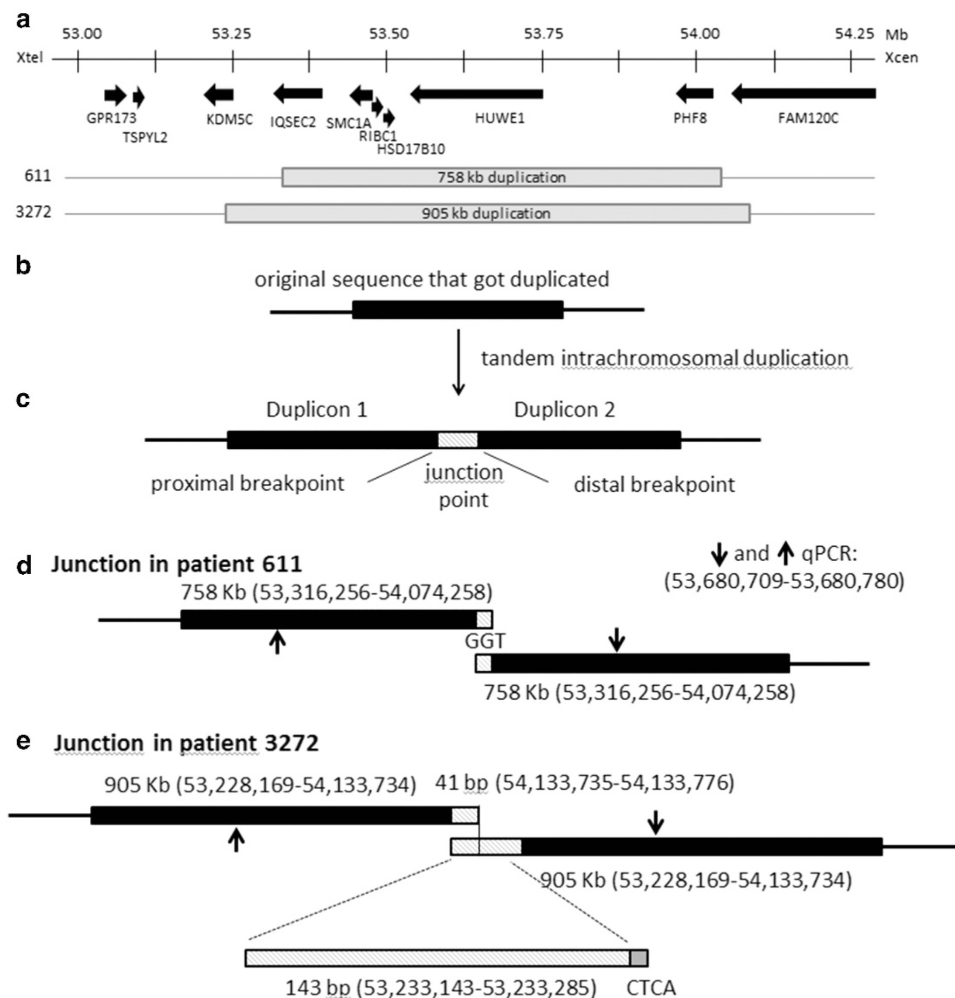

Figure 2 Representation of the Xp11.22 copy number gains in patients 611 and 3272. (**a**) Genomic schematic view of the duplications along the X chromosome and involved genes. (**b**) Wild-type Xp11.22 segment. (**c**) Scheme of the tandem intrachromosomal duplications. (**d**) Junction point sequences in patient 611, in which the duplication is 758 kb in size. A microhomology of three nucleotides (GGT) is present at the junction. (**e**) Junction point sequences in patient 3272. The junction of the 905 kb duplication is more complex. A 143-bp segment from within the duplicated region, representing a short intronic stretch of KDM5C sequence, is inserted in between the two duplicons. Proximal breakpoint uses a 41-bp homologous segment to join the proximal part with the intermediate one. At distal breakpoint, an inserted four-nucleotide sequence (CTCA) joins the intermediate with the distal side of the duplication. Arrows show the region amplified by qPCR.

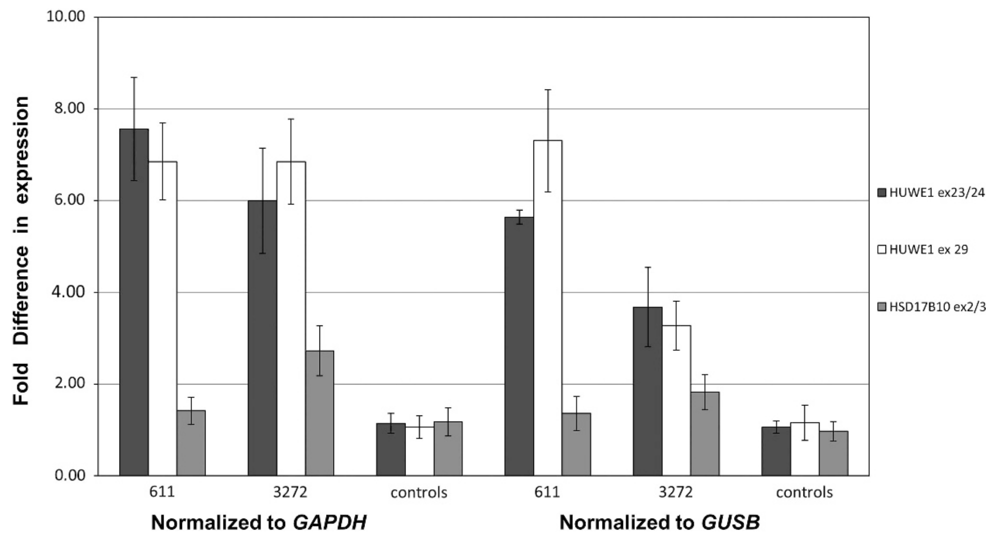

Figure 3 cDNA quantitative analysis of *HUWE1* and *HSD17B10* genes in patients 611 and 3272 in comparison with age-matched male controls. Expression levels were calculated relative to the mean levels obtained in the control samples by the ΔΔCt method, using the glucuronidase, beta *(GUSB)* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH)* genes as normalizers (fold difference; *y* axis). S.d. of three independent experiments were indicated for each bar.

Sequencing the breakpoint junction in patient 611 revealed microhomology of three nucleotides (GGT) at the proximal and distal sides of the duplication (Figure 2). In patient 3272, however, the rearrangement is more complex with a 143-bp intrachromosomal sequence (ChrX:53,233,143-53,233,285) inserted between the duplicons. This intermediate fragment is integrated at the proximal breakpoint through a 41-bp homologous segment (ChrX:54,133,735-54,133,776), whereas the breakpoint at the distal region had an insertion of four nucleotides (CTCA) (Figure 2). Bioinformatic analysis[2] demonstrated that the proximal breakpoints in both patients are not within a repetitive element, whereas the distal breakpoint in patient 611 is located inside a LINE/L2 (L2A) element and within an AluSx element in patient 3272. In patient 611, the mechanism underlying the duplication is consistent with the replication-based microhomology-mediated break-induced replication as proposed for most of the rearrangements encompassing *HUWE1*. [2] In patient 3272, however, the complex junction suggests a replication-based repair mechanism in which the microhomologous segment could have facilitated the template switching, serving as the priming site in the second replication fork. Nonetheless, the insertion of the CTCA sequence at the distal region might represent an information scar pointing to microhomology-mediated end joining, an alternative pathway to non-homologous end joining. While the presence of microhomology is optional to non-homologous end joining, it is a requirement to microhomology-mediated end joining.[8] Besides, microhomology-mediated end joining uses longer stretches of microhomology than those used in non-homologous end joining. Taken together, the duplication in patient 3272 could have resulted from either replication-based repair or microhomology-mediated end joining pathways.[9]

To infer about the possible origin of the duplication events, we analyzed high polymorphic SNPs and repeat markers within the duplicated regions.[2] As the grandmothers as well as carrier mothers were heterozygous for at least one of these SNPs or marker, and the male patients were not heterozygous for these positions (Supplementary Table 2), the recombination event should have been intrachromosomal.

Finally, mRNA expression analysis in blood cells of our two patients exhibited a *HUWE1* overexpression of three- to sevenfold (Figure 3). These values are strikingly higher than those observed in the families in which the analysis was done starting from Epstein–Barr virus-transformed peripheral blood lymphocytes.[1,2] These data are in agreement with those observed in the proband of family FAM3 that also started from blood lymphocytes to assess the *HUWE1* expression levels.[1]

In conclusion, increased expression of *HUWE1* owing to genomic duplications at Xp11.22 causes mild-to-moderate ID with a highly diverse array of additional clinical features.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)