Severe Neurological Phenotype in a Girl with Xp22.31 Triplication

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Established Facts

- The Xp22.31 duplication seems to be a risk factor for neurobehavioral phenotypes, but little is known about the clinical significance of the triplication.

Novel Insights

- Our patient confirms that the Xp22.31 triplication is more penetrant than the duplication with a neurological phenotype.

Keywords

Copy number variants · Infantile spasms · Intellectual disability · Xp22.31 gain

Abstract

The Xp22.31 duplication is a copy number variant which is challenging to categorize as pathogenic or benign. There is an increasing number of patients with the duplication and a neurobehavioral phenotype, but the duplication is almost always inherited from a parent, who in some cases is phenotypically normal. Also, the duplication is detected in the general population, though in a smaller percentage than in clinically ascertained populations. The Xp22.31 triplication has only been identified in 3 individuals of a large cohort of developmental delay cases but never in the control cohorts or general population. We report a severely affected female

with an Xp22.31 tetrasomy, inherited from duplications identified in both phenotypically normal parents. Although our study has limitations, it suggests that the Xp22.31 triplication seems to be more penetrant than the duplication and is associated with a neurological phenotype.

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The distal portion of the short arm of the human X chromosome is a region with genomic instability [Liu et al., 2011]. Xp22.31 deletions are associated with X-linked ichthyosis (OMIM 308100), developmental delay, and attention deficit hyperactivity disorder (ADHD) [Ballabio et al., 1987; Van Esch et al., 2005; Kent et al., 2008]. Xp22.31 duplications, the counterpart of the Xp22.31 deletion causing X-linked ichthyosis containing the *STS* gene, are among the most frequent findings in genetic

laboratories but are challenging to interpret [Shaffer et al., 2007; Li et al., 2010; Furrow et al., 2011; Liu et al., 2011; Faletra et al., 2012; Esplin et al., 2014]. This recurrent duplication contains 4 known genes, *PUDP*, *STS*, *VCX*, and *PNPLA4* as well as 2 microRNAs, *MIR651* and *MIR4767*. There is a high incidence of this duplication in clinical cases referred for array comparative genomic hybridization (aCGH) and ascertained due to neurobehavioral abnormalities, but the duplication is also observed in the general population. In the majority of clinical cases, it is inherited from a parent, most of them clinically unaffected [Li et al., 2010; Furrow et al., 2011; Liu et al., 2011; Faletra et al., 2012; Esplin et al., 2014]. The duplication may predispose to disease, but the manifestation may require additional genetic and/or environmental factors.

With these considerations, the Xp22.31 triplication must be more penetrant for the phenotype. We have only found 3 triplication cases in the literature, all with developmental delay, and in the 2 male individuals, aggressive behavior with features of ADHD was also described [Liu et al., 2011]. The triplication, as far as we know, has not been identified in the general population reinforcing the potential pathogenicity.

Here, we describe a severely affected female with an Xp22.31 tetrasomy, inherited from duplications that have been identified in both parents.

Patient and Methods

The girl was born at term by spontaneous vaginal delivery after an uneventful first pregnancy. Both parents are healthy with a normal phenotype. The mother, 26 years old, and the father, 37 years old, were born in Morocco and are first cousins. The 1-min Apgar score was 9; the 5-min score was 10. Her birth weight was 2,760 g (19th centile), length was 47 cm (11th centile), and her occipitofrontal circumference (OFC) was 34 cm (48th centile).

The neonatal period was unremarkable, but a developmental delay was detected in the first months. In a neuropsychiatric evaluation at 6 months of age, a severe axial hypotonia was present. The girl could not hold her head, the postural reflexes were absent with persistence of archaic ones, and pyramidal signs were present in her limbs. She was diagnosed with cerebral palsy (spastic quadriplegia). There was also a decrease in OFC from the 48th centile at birth to the 4th centile at 6 months of age. No facial dysmorphism was identified in the patient.

A brain MRI at 10 months showed symmetric and bilateral supra- and periventricular leukodystrophy, also affecting the temporal horns but being less intense in the dentate nucleus (Fig. 1). The EEG showed generalized slow waves. The ophthalmological evaluation, visual evoked potentials, and metabolic study were normal. The karyotype of the patient was 46,XX. aCGH (180K Agilent® array) identified an interstitial 1.6-Mb Xp22.31 tetrasomy, arr[hg

Fig. 1. Cranial magnetic resonance images showing coronal (A) and sagittal planes (B) of the patient at 10 months of age with symmetric and bilateral supra- and periventricular leukodystrophy (arrows), also affecting the temporal horns but being less intense in the dentate nucleus.

18] Xp22.31(6,477,066–8,091,810)×4 (Fig. 2A), containing the *PUDP*, *STS*, *VCX*, and *PNPLA4* genes as well as 2 microRNAs, *MIR651* and *MIR4767*. There were no additional copy number variants (CNVs) identified as causative or of unknown significance in the aCGH. Both parents showed the same Xp22.31 microduplication in their arrays (Fig. 2B).

The patient, now 2 years old, developed infantile spasms controlled with antiepileptic monotherapy and is also on baclofen treatment for spasticity. She has a severe developmental delay with no language, is unable to sit independently, attends a rehabilitation program, and wears orthoses.

Discussion

Since the clinical use of aCGH techniques, numerous pathogenic CNVs have been identified, but some of them are difficult to categorize as pathogenic or benign despite careful analysis.

Currently, there is a debate about the pathogenicity of the Xp22.31 duplication [Li et al., 2010; Furrow et al.,

Fig. 2. **A** aCGH of the patient identified an interstitial 1.6-Mb Xp22.31 tetrasomy, red line with the gain (4 copies): arr[hg 18] Xp22.31(6,477,066–8,091,810)×4, containing the *PUDP*, *STS*, *VCX*, and *PNPLA4* genes as well as 2 microRNAs. **B** DNA hybridization of the father and the mother of the same Xp22.31 region where the log₂ ratio values show that both parents have a microduplication of the same region (ratio 2 vs. 3 copies with 1 vs. 2 copies in the rest of the X chromosome).

2011; Liu et al., 2011; Faletra et al., 2012; Esplin et al., 2014]. An increasing number of patients with the duplication share a common neurobehavioral phenotype. The variability in the expression may be related to the impact of reduced penetrance, the different genes in the duplicated region, a position effect, and X inactivation, among other modifiers. Data [Li et al., 2010; Liu et al., 2011] show that skewed X inactivation is not directly associated with the manifestation of an abnormal phenotype because the majority of affected females and healthy mothers revealed random or noninformative patterns of X inactivation.

Studies [Li et al., 2010; Furrow et al., 2011; Liu et al., 2011; Esplin et al., 2014] have shown, and these argue against the potential pathogenicity, that the duplication is almost always inherited, usually from the mothers, with the absence of abnormal phenotypes in some carriers, although the possibility of an unnoticed phenotype in carrier parents exists. Regarding the gender of the reported cases, except Furrow et al. [2011], who reported only males, the studies of Li et al. [2010], Liu et al. [2011], and Faletra et al. [2012] identified both males and females with no significant gender differences.

Moreover, the duplication is also detected in the general population, though in a smaller percentage than in clinically ascertained population, in the majority of the published cases in literature [Li et al., 2010; Liu et al., 2011; Faletra et al., 2012]. The duplication can be a risk factor for neurobehavioral complications that triggers the phenotype if additional factors are present; these modifiers can be environmental, genetic, and/or epigenetic factors.

A previous study has given evidence suggesting that an increased copy number of genes on the X chromosome can contribute to a dysregulation of normal cognitive development [Froyen et al., 2007], and that a position effect could be implicated as it has been reported in several other human genetic conditions associated with chromosomal rearrangements [Velagaleti et al., 2005], making it a possible mechanism for expressing the phenotype.

The majority of Xp22.31 duplication individuals carry a 1.6-Mb common recurrent duplication flanked by 2 low-copy repeats, which is the apparent reciprocal rearrangement to the previously reported recurrent deletion [Yen et al., 1990], and a nonallelic homologous recombination.

nation (NAHR) [Van Esch et al., 2005] is the implicated mechanism, but there are also cases with apparently simple nonrecurrent duplications of different sizes, and apparently complex rearrangements, where other mechanisms are implicated [Liu et al., 2011].

Only 1 reference with a large cohort of developmental delay cases ($n = 20,095$) identified an Xp22.31 triplication in 3 individuals, 2 males and 1 female [Liu et al., 2011]. The description of the clinical phenotypes includes 100% developmental delay with gross motor and speech delay, aggressive behavior in both males with features of ADHD, hypotonia in 1 male and the female, macrocephaly in the female, gastroesophageal reflux in 1 male as well as short stature in 1 male. No triplication is found in the 5,088 individuals of the control cohort of this reference [Liu et al., 2011], nor in the control cohort of the other 2 studies [Li et al., 2010; Faletra et al., 2012].

The 3 triplications described previously [Liu et al., 2011], and also our case, have a similar size and extent as the common recurrent duplication that seems to be generated by a NAHR event. In our case, where the triplication is inherited from both consanguineous parents with the duplication, we cannot exclude an identical-by-descent origin of the duplication. Information on the family history was not available.

Among the genes included in the triplication region of our patient, there are some that can contribute to a neurological phenotype. *STS* encodes steroid sulfatase, an enzyme that hydrolyzes precursors that are neurosteroids affecting neurophysiological and behavioral functioning [Davies et al., 2009]. The deficit has been associated with

intellectual disability, ADHD, autism, as well as disorders of social communication [Van Esch et al., 2005; Brookes et al., 2008; Kent et al., 2008], and cause X-linked ichthyosis (OMIM 308100) [Ballabio et al., 1987]. *VCX* is involved in the regulation of neuritogenesis [Jiao et al., 2009]. Other genes in the affected region need further study.

The patient described here, and the 3 previously reported cases, show that the triplication seems to be more penetrant than the duplication with respect to an association with abnormal phenotypes. In our study, there are some limitations about the interpretation of the phenotype due to the possibility of other influencing factors, moreover given the consanguinity of the case. We can exclude additional CNVs by aCGH analysis. However, regarding that no other genetic studies were performed, we cannot exclude further genetic factors contributing to the patient's phenotype, mainly additional homozygous variants undetectable by aCGH or by the X-inactivation status in the patient.

Statement of Ethics

The procedures performed were in accordance with the ethical standards.

Disclosure Statement

The authors declare no conflicts of interest.

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