Xp22.33p22.12 Duplication in a Patient with Intellectual Disability and Dysmorphic Facial Features

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Key Words

Duplication Xp22.33p22.12 · Dysmorphic facial features · Intellectual disability

Abstract

A novel 19.98-Mb duplication in chromosome Xp22.33p22.12 was detected by array CGH in a 30-year-old man affected by intellectual disability, congenital hypotonia and dysmorphic features. The duplication encompasses more than 100 known genes. Many of these genes (such as neuroligin 4, cyclin-dependent kinase like 5, and others) have already correlated with X-linked intellectual disability and/or neurodevelopmental disorders. Due to the high number of potentially pathogenic genes involved in the reported duplication, we cannot correlate the clinical phenotype to a single gene. Indeed, we suggest that the resulting clinical phenotype may have arisen from the overexpression and consequent perturbation of fine gene dosage.

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X-linked mental retardation is a common clinically complex and genetically heterogeneous condition arising from mutations and/or copy number variants in many

genes located in the X chromosome [Ropers et al., 2005]. Xp duplications have been described in males affected by intellectual disability and variable congenital abnormalities [Honda et al., 2010; Salaria et al., 2012; Wu et al., 2013].

We hereby report on a 30-year-old man with a de novo 19.98-Mb duplication in chromosome Xp22.33p22.12 affected by severe intellectual disability, congenital hypotonia and dysmorphic features, including prominent glabella, short philtrum, micrognathia, high forehead, hypertelorism, epicanthal folds, downslanting palpebral fissures, wide nasal bridge, and short upper lip.

Case Report

The proband, an adult male now 30 years old, was born at 38 weeks of gestation by spontaneous delivery from nonconsanguineous and healthy parents. Prenatal data are not available. His birth weight was normal being 3,300 kg (50th centile). No intrauterine or postnatal growth delay was recorded. At 6 months, hypotonia and microcephaly with craniostenosis were diagnosed, but at 4 years of age, cranial growth had partly recovered, and his head circumference was normal by the age of 9. Bilateral cryptorchidism was corrected surgically at 3 years of age. Physical examination identified dysmorphic features, including prominent glabella, short philtrum, micrognathia, high forehead, hypertelorism,

Fig. 1. The patient at 30 years old. **a** Skeletal features include thin legs, right radioulnar synostosis, valgus deformity, and talipes equinovarus. **b** Dismorphic facial features include prominent glabella, short philtrum, high forehead, hypertelorism, epicanthal folds, downslanting palpebral fissures, wide nasal bridge, and short upper lip.

epicanthal folds, downslanting palpebral fissures, wide nasal bridge, ogival palate, short upper lip, and hypodontia (fig. 1). Some, though not all developmental milestones were delayed: he walked unsupported at 24 months, but expressive language for single words began at about 16 months. Hearing acuity was found throughout his life and was tested approximately every 2 years. Mild visual anomalies were present including astigmatism and hyperopia diagnosed at age 5 and 13, respectively. Cardiac and abdominal sonograms were normal during childhood, but by 17 years of age he developed a mitral and tricuspid valve prolapse as well as prominent varicose veins. His bone age was normal at 6 years, but scoliosis developed during adolescence. Other skeletal features include thin legs, right radioulnar synostosis, valgus deformity, and talipes equinovarus (fig. 1). The patient's nail growth is absent, tooth eruption was delayed and some permanent teeth never erupted. Classical G-banding, EEG, MRI, and CT scans were all normal, reporting only a slight asymmetry of the lateral ventricles. Neurological and neuropsychological deficits currently include severe intellectual disability (Coloured Progressive Matrices score: 17/36, <10th percentile), attention deficit/hyperactivity disorder with only attention deficits and no hyperactivity, verbal and cognitive perseverative behavior, ideomotor apraxia with difficulty in recognizing and imitating symbolic gestures, poor visuomotor integration and bimanual coordination, severe oropharyngeal dyspraxia with phonological deficits and swallowing disorders, hyposthenia, and hypereflexia. Additional deficits include (a) graphic perseveration and/or impairment in spatial abilities with visuocstructional apraxia (Rey complex figure score: 4/36; <10th percentile), (b) visual executive functions (clock-drawing test and trail-making test), and (c) phonological fluency (no words produced for 3 phonemic cues in the controlled oral

word association test) and naming pictured objects (Boston naming test score: 34/60). Functions found within the normal range, following the correction for age and education level include the recognition of incomplete and fragmented figures (Street test: 7/14) and recalling a list of words when tested by an additional recognition trial (Rey auditory verbal learning scores for immediate recall = 14.1/75; for delayed recall = 3.4/15; for recognition = 12/15). The patient's better recognition score compared to the immediate and delayed recall scores are compatible with a specific deficit in information retrieval, while information storage is relatively spared. Since his childhood, the patient underwent consecutive periods of rehabilitation, especially speech therapy. This may have contributed to the production of words semantically correlated with the target picture and of gestures appropriated for the use of an object. His parents also reported a specific ability in learning the names of some word categories (e.g. car models). Periodic reports from the rehabilitation team retrospectively document a slow, but constant improvement over time in many areas including social, communicative, motor, and language skills. Despite this improvement, severe intellectual disability and dysmorphic features persisted into adulthood. As an adult, he is interested in music, photography, voluntary work, and singing. He likes to help in simple household jobs, has a friendly attitude and makes contact easily. As a child, he was diagnosed with a mild form of Wolf-Hirschhorn syndrome (WHS) without a typical WHS 4p16.3 deletion [Zollino et al., 1996] and without intrauterine and postnatal body growth delay which is a core feature of WHS [Moretti et al., 2001]. Indeed, the facial resemblance between our patient and patients with classical WHS are no longer pronounced, suggesting that this patient had a developmental trajectory bringing him outside of the WHS phenotype.

Fig. 2. The 20-Mb duplication in chromosome band Xp22.33p22.12 between 61,091 and 20,038,601 bp (Build 37: February 2009) detected using SurePrint G3 Human CGH 4x180K Microarray kit.

Methods

Array CGH was performed using the SurePrint G3 Human CGH 4x180K Microarray kit (Agilent Technologies, Santa Clara, Calif., USA) on genomic DNA from blood. The duplication was confirmed using a TaqMan Copy Number Assay targeting intron 3 of the retinoblastoma-binding protein 7 (*RBBP7*) gene and normalized with the *RNase P* gene. RNA was extracted from peripheral blood mononuclear cells, retrotranscribed into cDNA and used to quantify *RBBP7* mRNA level by TaqMan methodology; expression values were normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), used as an internal control. Network analysis was performed using the web tool 'STRING' 9.1 [Franceschini et al., 2013].

Results and Discussion

The patient is carrier of a de novo duplication located in chromosome Xp22.33p22.12, spanning 19.98 Mb and encompassing more than 100 genes (fig. 2; table 1). The

duplicated region started from oligomer A_18_P17041097 (61,091 bp, first deleted) to oligomer A_16_P03666848 (20,038,601 bp, last deleted). A total of 1,856 gains and 54 losses have been reported in this region in the Database of Genomic Variants [MacDonald et al., 2013], each overlapping well below 10% with the present aberration. The Xp duplication harbors several genes classified as 'disease genes' in the OMIM database of genetic disorders (<http://www.omim.org/>). Single nucleotide mutations and/or deletions in some of these genes have been associated with neurodevelopmental disorders including neuroligin 4 (*NLGN4*; OMIM 300427, associated with autism, intellectual disability, Asperger syndrome), cyclin-dependent kinase like 5 (*CDKL5*; OMIM 300203, associated with Rett syndrome, epileptic encephalopathy), orofacial digital syndrome I (*OFD1*; OMIM 300170, associated with Joubert syndrome characterized by hypotonia, cerebellar ataxia and developmental delay), and adaptor-related protein complex 1 sigma 2 subunit (*AP1S2*; OMIM

Table 1. Comparison of clinical features observed in our patient and in patients with overlapping Xp22.31p22.12 duplication

Locus	Size	Phenotype	Genes	Reference
Xp22.33p22.12	19.98 Mb	intellectual disability, congenital hypotonia, dysmorphic features	<i>PLCXD1, GTPBP6, NCRNA00107, PPP2R3B, SHOX, CRLF2, CSF2RA, IL3RA, SLC25A6, NCRNA00105, ASMTL, P2RY8, SFRS17A, ASMT, DHRSX, ZBED1, CD99, XG, XGPY2, GYG2, ARSD, ARSE, ARSH, ARSF, MXRA5, PRKX, NLGN4X, VCXA, HDHD1A, STS, VCX, PNPLA4, MIR651, VCX2, VCX3B, KALI, FAM9A, FAM9B, TBL1X, GPR143, SHROOM2, WWC3, CLCN4, MID1, HCCS, ARHGAP6, AMELX, MSL3, FRMPD4, PRPS2, TLR7, LOC349408, TLR8, TMSL3, TMSB4X, FAM9C, ATXN3L, EGFL6, TCEANC, RAB9A, TRAPPC2, OFD1, GPM6B, GEMIN8, GLRA2, FANCB, MOSPD2, ASB9, ASB11, PIGA, FIGF, PIR, BMX, ACE2, TMEM27, CA5BP, CA5B, INE2, ZRSR2, AP1S2, GRPR, CTPS2, S100G, SYAP1, CXorf15, RBBP7, REPS2, NHS, SCML1, RAI2, BEND2, SCML2, CDKL5, RS1, PPEF1, LOC100132163, PHKA2, GPR64, PDHA1, MAP3K15, SH3KBP1, CXorf23, LOC729609, MAP7D2</i>	present case
Xp22.31p22.2	2.8 Mb	ichthyosis, congenital heart defect, varicose veins, speech delay, hypogonadotropic hypogonadism	<i>STS, VCX, PNPLA4, MIR651, VCX2, VCX3B, KALI, FAM9A, FAM9B, TBL1X, GPR143, SHROOM2, WWC3</i>	Salaria et al., 2012
Xp22.2	737 kb	West syndrome, severe mental retardation, epilepsy, absent speech	<i>REPS2, NHS</i>	Honda et al., 2010
Xp22.2	737 kb	moderate mental retardation, speech delay, autistic features	<i>REPS2, NHS</i>	Honda et al., 2010
Xp22.2	632 kb	mild mental retardation, autism	<i>ARHGAP6, FRMPD4, MSL3</i>	Honda et al., 2010
Xp22.33	290 kb	intellectual disability	<i>SHOX</i>	DECIPHER, 262464
Xp22.33	982 kb	global developmental delay	<i>SHOX, CRLF2, CSF2RA, IL3RA</i>	DECIPHER, 294106
Xp22.33p22.32	2.2 Mb	autism	<i>AK000470, AK000470, AK097803, ARSD, ARSE, ARSF, ARSH, CXorf28, GYG2, LOC389906, MXRA5, PRKX, XG</i>	DECIPHER, 294320
Xp22.31	1.57 Mb	global developmental delay, abnormal facial shape	<i>HDHD1A, STS, VCX, PNPLA4, MIR651</i>	DECIPHER, 280977
Xp22.33	807 kb	delayed speech and language development, intellectual disability, short stature	<i>SHOX, CRLF2, CSF2RA</i>	DECIPHER, 289637
Xp22.33	1.5 Mb	intellectual disability	<i>HDHD1A, STS, VCX, PNPLA4, MIR651</i>	DECIPHER, 251816

300629, associated with intellectual disability). Other OMIM genes correlated with known disorders, but not directly to neurodevelopment disorders, include short stature homeobox (*SHOX*; OMIM 312865, associated with Langer mesomelic dysplasia), steroid sulfatase (*STS*; OMIM 308100, associated with ichthyosis), arylsulfatase E (*ARSE*; OMIM 300180, associated with chondrodysplasia punctata), Kallmann syndrome interval gene 1 (*KALI*; OMIM 300836, associated with hypogonadotropic hypogonadism), midline 1 (*MID1*; OMIM 300552, associated with Opitz GBBB syndrome type I), amelogenin (*AMELX*; OMIM 300391, associated with amelogenesis imperfecta type 1E), Nance-Horan syndrome gene (*NHS*; OMIM

300457, associated with cataract), retinoschisin (*RS1*; OMIM 300839, associated with retinoschisis), phosphor-ylase kinase liver alpha-2 subunit (*PHKA2*; OMIM 300798, associated with glycogen storage disease), and pyruvate dehydrogenase alpha-1 (*PDHA1*; OMIM 300502, associated with pyruvate dehydrogenase E1-alpha1 deficiency). In addition, many other genes within the duplication play an important role in the nervous system and have been found to be associated with neurodevelopmental disorders. The ferm and PDZ domains containing the protein 4 gene (*FRMPD4*), a positive regulator of dendritic spine morphogenesis and density, has been found mutated in patients affected by intellectual disability.

ity [Rauch et al., 2012], autism and schizophrenia [Piton et al., 2011]. Similarly, de novo mutations in the neuronal synapse-associated protein 1 gene (*SYAP1*) and in the glycine receptor alpha-2 subunit gene (*GLRA2*), a ligand-gated channel mediating a chloride-dependent inhibitory neurotransmission, have been found, respectively, in patients with autistic disorder [Prasad et al., 2012] and in patients affected by autism and schizophrenia [Piton et al., 2010]. A search for other patients with an Xp22.33p22.12-duplicated chromosome in the literature and in the Database of Chromosomal Imbalance and Phenotype in Humans DECIPHER (<http://decipher.sanger.ac.uk>) revealed many patients with even much smaller duplications in this chromosome location (table 1). The DECIPHER database reports ~300 patients with overlapping duplications ranging in size from 2 kb to 155 Mb. However, many of these patients, especially those with larger duplications, harbor additional chromosomal imbalances in their genome and, therefore, cannot be directly compared to our patient. Table 1 summarizes patients carrying unique Xp22.33p22.12 rearrangements. All DECIPHER patients (262464, 264106, 294320, 280977, 289637, and 251816) have intellectual disability and/or neurodevelopmental disorders including autism, delayed speech or language development and dysmorphic features. The patient described by Salaria et al. [2012] harbors a 2.8-Mb Xp22.31p22.2 duplication in his genome: he had speech delay, ichthyosis, congenital heart defects, hypogonadotropic hypogonadism, varicose veins, and hypotonia. The 2 latter anomalies were also present in our patient. Patients with an Xp22.2 duplication [Honda et al., 2010] have mental retardation ranging from mild to severe and/or autistic traits and epilepsy (table 1).

Although many of the dysmorphic features presented in our case are relatively nonspecific, a mild form of WHS was clinically diagnosed as a child [Zollino et al., 1996]. This initial diagnosis questions whether any gene spanned by the present duplication may functionally interact with the Wolf-Hirschhorn candidate gene 1 (*WHSC1*) located in chromosome 4p16.3, so as to possibly explain the initial WHS-like features with a relatively favorable developmental trajectory. According to network analysis, the only interaction between any duplicated gene and *WHSC1* involves *RBBP7*, which encodes for the retinoblastoma binding protein 7, a transcriptional regulator. STRING [Franceschini et al., 2013] detects protein-protein interactions supported by experimental evidence and mediated by histone deacetylase 1 (*HDAC1*). These interactions lead to predict that a gain of an extra *RBBP7* allele should functionally equate a loss in a *WHSC1* allele in reference to

Fig. 3. Relative level of *RBBP7* transcript in leukocytes of the proband (PB), compared to 4 unaffected controls (C1–C4). The expected $\Delta\Delta C_t$ ratio is 0.5, 1, and 1.5 when 1, 2 or 3 allele copies are expressed, respectively.

HDAC1 activity. RT-qPCR expression analysis shows increased *RBBP7* mRNA levels, consistent with the presence of the genomic duplication in the proband's leukocytes (p values range from 2.5×10^{-5} to 1.9×10^{-8} in different control individuals; fig. 3). Instead, no differences in *HDAC1* and *WHSC1* mRNA levels were detected between the proband and controls (data not shown), confirming the post-transcriptional nature of these interactions. Cellular and animal models will be necessary to ultimately address the functional roles of *RBBP7* in clinical conditions sharing dysmorphic features resembling WHS during infancy.

The potential clinical relevance of the extended chromosomal alteration in our patient is supported by the numerous disease genes found within the duplicated Xp22.33p22.12 region. As previously mentioned, duplications of the Xp chromosome have been associated with neurodevelopmental disorders [Honda et al., 2010; Salaria et al., 2012; see DECIPHER website]. Due to the 'Lyon hypothesis', females with X chromosome duplications are generally less severely affected than males [Matsuo et al., 1999]. However, females with a defect in the X chromosome inactivation mechanism show severe mental retardation, developmental delay, dysmorphic facial features, and heart defects [Migeon et al., 1993]. These females somehow resemble our patient. Due to the large size of the Xp duplication in our case, it is difficult to assign a causal role to a specific gene or set of genes. Nonetheless,

since the present Xp22.33p22.12 duplication harbors more than 100 genes, overexpression of only some of them may have influenced the clinical phenotype, either directly or through an indirect dysregulation of genes located in other chromosomes, as demonstrated for Down syndrome [revised in Lintas et al., 2012]. These scenarios will have to be explored using genome-wide transcriptomic analysis.

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Statement of Ethics

Written informed consent was obtained from the patient's parents for publication of this case report and any accompanying images. The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflict of interest to declare.

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