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Case Report

Molecular characterisation of a der(Y)t(Xp;Yp) with Xp functional disomy and sex reversal

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ABSTRACT. Sex reversal due to duplication of the Xp21 dosage-sensitive sex reversal locus results in XY females with gonadal dysgenesis. Pure Xp disomy (without a concurrent loss of genetic material) can occur by translocation or interstitial duplication. The case reported here is the rare form with a t(Xp;Yp). The combination of conventional clinical cytogenetic techniques, microsatellite analysis and high-density microarrays identified the X-chromosome breakpoint as centromeric of the *NR0B1* gene and its control elements. Cytogenetics and array technology complemented each other in characterizing the translocation event and the extent of the dosage-sensitive sex reversal critical region on the derivative Y-chromosome. The implications of this analysis also lie in genetic counseling that highlight the likely *de novo* nature of a paternal meiotic event.

Key words: Xp functional disomy; Duplication Xp; Sex reversal; Translocation X;Y; Microarray

INTRODUCTION

Gonadal development is bipotential during the first 7-8 weeks of mammalian embryogenesis. Subsequent differentiation occurs to give rise to testes or ovaries in individuals with a 46,XY or 46,XX karyotype, respectively. The *SRY* gene plays a pivotal role in testis determination, but several other genes encode for proteins that are required for gonad development. These genes include *DHH*, *LHCGR*, *SF1*, *WT1*, *WNT4*, *NR0B1* (*DAX1*), and *SOX9*. Mutations in these genes can lead to gonadal disorder of sex development (Table 1).

Table 1. Genes other than *SRY* that are implicated in gonadal disorder of sex development.

Gene	Duplications	Haploinsufficiency
DHH		Gonadal dysgenesis +/- polyneuropathy: homozygous point mutation (Umehara et al., 2000; Canto et al., 2004)
LHCGR		Leydig cell hypoplasia and under-virilization in 46,XY male, hypergonadotropic hypogonadism in 46,XX females: heterozygous inactivating mutations (Chan, 1998)
SF1		Gonadal dysgenesis/agenesis, +/- adrenal insufficiency, 46,XY sex reversal: heterozygous point/microdeletion mutation (Achermann et al., 1999; Correa et al., 2004; Mallet et al., 2004; Hasegawa et al., 2004; Lin et al., 2007; Kohler et al., 2008); homozygous point mutation (Achermann et al., 2002).
WT1		Gonadal dysgenesis, 46,XY sex reversal: heterozygous deletion (Le Caignec et al., 2007)
WNT4	46,XY sex reversal (Jordan et al., 2001)	Absence of uterus and androgen excess, 46,XX female: dominant negative loss of function mutation (Biason-Lauber et al., 2004, 2007)
NR0B1 (DAX1)	Gonadal dysgenesis, 46,XY sex reversal (Bardoni et al., 1994)	Congenital adrenal hypoplasia and hypogonadic hypogonadism (Zanaria et al., 1994; Muscatelli et al., 1994)
SOX9	46XX sex reversal (mosaicism of duplication; Huang et al., 1999)	Campomelic dysplasia and gonadal dysgenesis, 46,XY sex reversal: heterozygous point mutation (Foster et al., 1994; Cameron et al., 1996).

Of these genes, there are limited reports of individuals carrying duplications of the dosage-sensitive sex reversal (DSS) critical region on the X-chromosome that encompasses the *NR0B1* and *MAGEB* genes (Xp21.2-p21.3) (Barbaro et al., 2007). Duplication of this region, which would otherwise be subject to X-inactivation, is associated with isolated gonadal dysgenesis together with a range of extragonadal abnormalities (Barbaro et al., 2008). We report here a 46,X,+mar female carrying a rare unbalanced t(Xp;Yp) rearrangement, together with a high-resolution analysis of the X-chromosome breakpoint.

CLINICAL REPORT

The patient is the first child to a non-consanguineous couple. The mother's pregnancy was unremarkable until the last month when fetal growth retardation was documented. An ultrasound scan at that time demonstrated a hypoplastic corpus callosum and this was confirmed on a postnatal ultrasound. The child was born at term weighing 2.83 kg. She was formula fed and gained weight slowly. At four months of age, a brain magnetic resonance scan showed diffuse thinning of the corpus callosum and in particular the splenium was markedly hypoplastic. Prominence of the lateral ventricles was also noted. At 8 months of age, she was still unable to sit independently. Examination at that time showed her length between the 10th and 25th percentile, weight just below the third percentile and head circumference between the 50th and 75th percentile. She had generalised hypotonia, but she was able to fix and follow and smile. In the facies, there was mild metopic ridging, a bulbous nasal tip, pinched nares with protuberant columella, prominence of the philtral pillars, and a small mouth with

normal palate and uvula. She had normal female external genitalia. When last assessed at 2 years of age, she was able to sit, but was not crawling or walking and was able to make simple noises. A pelvic ultrasound performed at 21 months of age demonstrated a normal appearing uterus with dimensions of 23 x 8 x 10 mm. Ovarian tissue was not definitely identified and no testicular tissue was identified. An exploratory laparatomy with removal of any gonadal tissue was advised but has not been performed yet.

Genetic analysis

Conventional chromosome analysis showed a 46,X,+mar karyotype (Figure 1). The marker chromosome was found to be a derivative Y originating from a t(Xp;Yp), using standard fluorescence *in situ* hybridisation (FISH) analysis. The breakpoint within Yp was distal to the *SRY* gene, and the additional Xp material was estimated to extend from Xpter to Xp21.

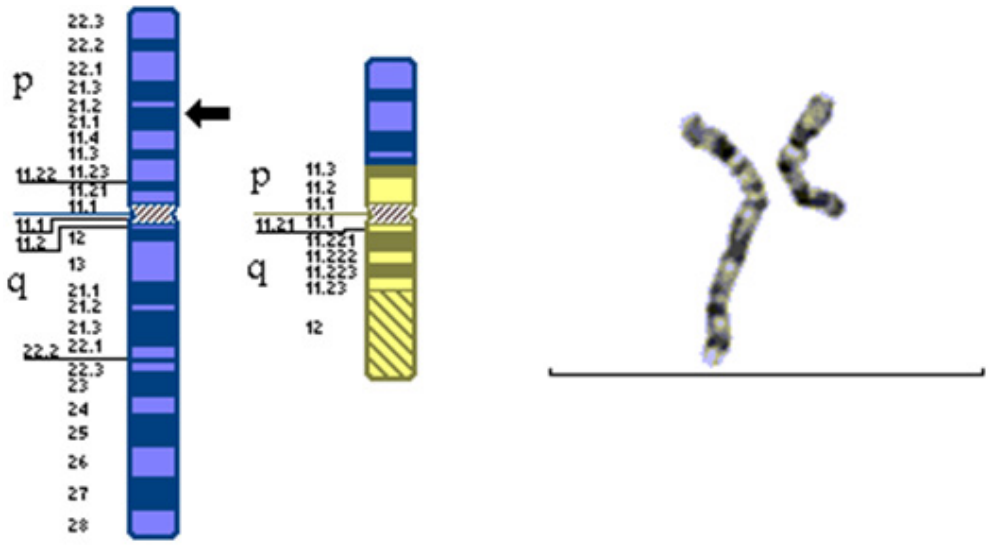

Figure 1. Ideograms and GTG-banded chromosomes showing the normal X-chromosome (left) and derivative Y-chromosome (right). The Xp21.1 breakpoint is marked by an arrow.

Following the result of the patient's 46,X,der(Y)t(X;Y)(p21.1;p11.3) karyotype, a number of Xp21.3-21.2 microsatellite loci were analysed to assess the extent of the duplicated X-chromosome region. Primers were used that had been designed against known sequence-tagged site markers; however, primers were also designed using the FastPCR programme (http://www.biocenter.helsinki.fi/bi/Programs/fastpcr.htm) against di- and tri-nucleotide repeats localised to a small genomic region encompassing the *NR0B1* gene (UCSC genome browser; http://genome.ucsc.edu/); see Table 2. All primers were assessed using SNPcheck (http://ngrl.man.ac.uk/SNPCheck/SNPCheck.html#) to confirm that they did not overlie single-nucleotide polymorphisms.

Table 2. X-chromosome microsatellite loci used for haploytpe analysis.

Microsatellite loci	Forward primer	Reverse primer	Start	End	Alleles (bp)
AFM292WB9/DXS1218	AAGACTAAGATTGTTTCAGTTTGTT	GTGCTTTTGTAATTTTTACCCA	29065244	29065510	267
GATA124E07/DXS9896	CCAGCCTGGCTGTTAGAGTA	ATATTCCTATATTCCATATGGCACA	29247081	29247279	229
AFM224ZF2/DXS1065	TAATCTGATCCCCCAACCTGC	GGGACATTGTGAATACAGG	29899855	29900016	158
AFMB277ZB1/DXS8049	TCCCCAGTACCCATGAAG	GCCCTCACCAGATGCAATCC	29960377	29960612	231
L2Xp21.2[a]	GTACCTGAGTTTCCAGCCTTCCAG	CCTTGGCCATTTAGAAGCTGCCA	30194045	30194516	469, 471
L3Xp21.2[a]	GAGACACACAAACTGGCCTTCAA	ATGTTCCTCGTCCTGTTGGGA	30234338	30234767	427, 431
L4-1Xp21.2[a]	GTTCCCTCTGTTACCTTGAGCCA	CTGTACATCAGAACTCCAGCAG	30246313	30246540	227
L5-1Xp21.2[a]	AAGTATGGACCAATGATATTGCTGG	CTGTGTCTTGAACTGAGTGGTG	30328311	30328570	251, 257
L6-2Xp21.2[a]	ATGAGAGCTGGAGCCACAGAA	GGCCAATGGATGGGAAGCTCCACC	30332426	30332591	155, 163
AFM112XF2/DXS985	TTTCCAAACTTCACTAAAAC	AAAATGTTGTTGTACTGGTC	30490886	30491022	125, 133
AFM184XG5/DXS992	AAGAATGGGACTCCATTTCA	GCTTATCCACTGGGACAGAA	30660238	30660440	196, 200

[a]These loci correspond to di- and tri-nucleotide repeats identified in the UCSC genome browser (http://genome.ucsc.edu).

The analysis of the extent of heterozygosity in our patient indicated that the proximal breakpoint on the X-chromosome lay further centromeric than DXS992, and indicated that the patient carried two copies of the *NR0B1* gene (accession No. NM_000475; chrX co-ordinates 30232460-30237416 bp according to the NCBI36/hg18 human genome assembly); see Figure 2.

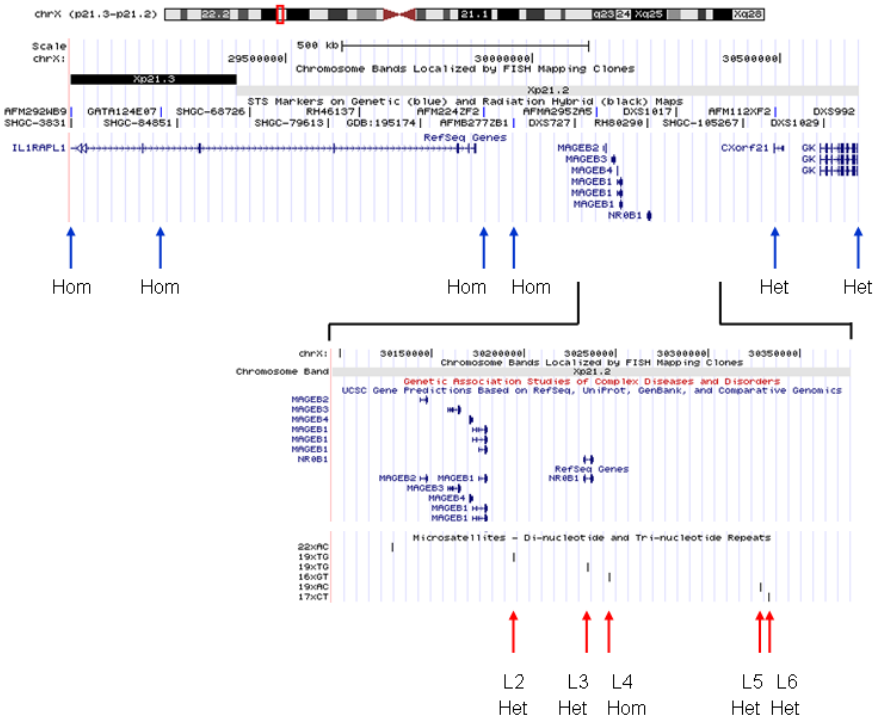

Figure 2. Localisation of Xpter microsatellites. Schematic taken from the UCSC genome browser (http://genome.ucsc.edu/) showing sequence-tagged site (STS) microsatellite loci that were used in this study (indicated by blue vertical arrows), together with an amplified view of the region encompassing the *NR0B1* gene showing di- and tri-nucleotide repeat loci (L2-L6); these loci are indicated by vertical red arrows. Homozygosity (Hom) and heterozygosity (Het) at the microsatellite loci for the patient described here are indicated.

To achieve more precision concerning the extent of the duplicated X-chromosome region, chromosomal microarray analysis (CMA) was performed on DNA extracted from peripheral blood using the Affymetrix® Cytogenetics Whole-Genome 2.7M Array, according to manufacturer instructions. Regions of copy number change were calculated using the Affymetrix Chromosome Analysis Suite software (ChAS) v.1.0.1.

Array analysis determined a complete complement of the male-specific Y-chromosome region and then confirmed the presence of the *SRY* gene identified by FISH. A large duplication of the distal Xp-region was estimated to comprise from Xpter to within Xp21.1 (chromosome co-ordinates X: 1-31,656,721). This region encompasses the *NR0B1* and *MAGEB* genes, and precisely mapped the proximal X-chromosome breakpoint to intron 52 of the dystrophin gene (Figure 3). The analysis of this intronic region shows repetitive elements such as *Alu*Y (CENSOR; Kohany et al., 2006; http://www.girinst.org/censor/index.php). Despite the resolution afforded by

the Affymetrix array used here, the data do not enable any conclusion to be made regarding the mechanism of the translocation event, which is likely to involve non-homologous end-joining as opposed to non-allelic homologous recombination (Erdogan et al., 2006; Chen et al., 2008).

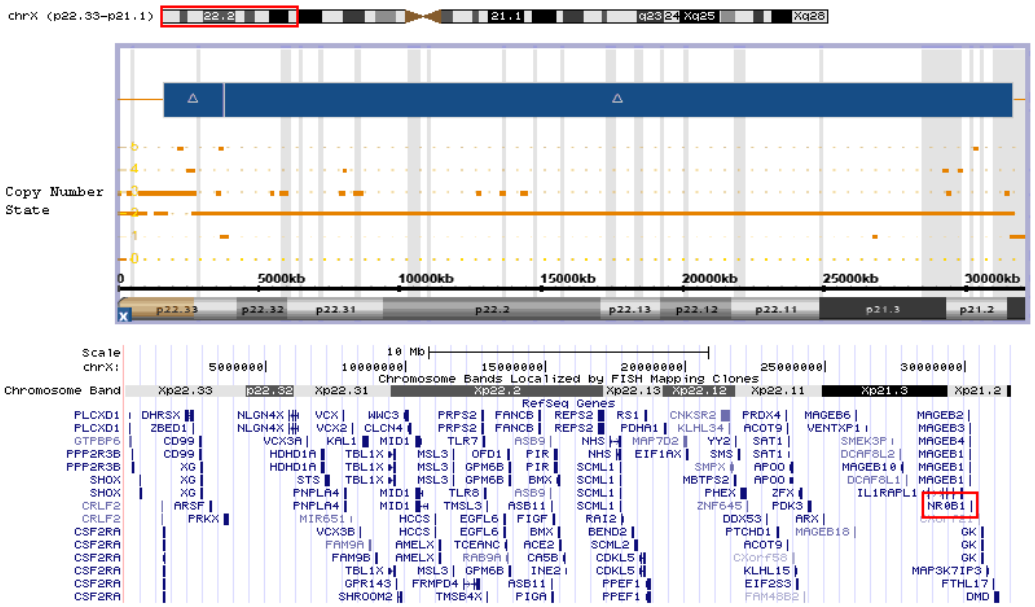

Figure 3. Duplicated region of Xp. Schematic taken from the UCSC genome browser (http://genome.ucsc.edu/) showing the extent of the duplicated region of Xp and the graphical output from ChAS analysis of the Affymetrix® array data. The *NR0B1* gene is indicated.

DISCUSSION

Sex reversal due to Xp21 disomy is rare and has been observed as a result of an interstitial duplication of Xp21, an X;A translocation or an X;Y translocation (Sanlaville et al., 2004; Barbaro et al., 2007, 2008; Tzschach et al., 2008). The patient reported here is one of a small number of reported cases resulting from an X;Y translocation (Table 3), and the only one that has been resolved at the level of oligonucleotide-based CMA. Four of these patients had female external genitalia, and one was ambiguous. Most had no identifiable gonadal tissue by ultrasound; one had streak gonads that went on to develop gonadoblastoma. All exhibited mental retardation, hypotonia, and dysmorphic facial features.

The extent of the extragonadal abnormalities observed in these individuals nominally relates to the size of the duplicated X-chromosome segment. However, the correlation of genotype and extragonadal phenotype is difficult in these cases due to the large number of duplicated genes involved and the absence of precise breakpoint characterisation. In addition, factors such as gene disruption or positional effects due to translocation may also play a role in phenotypic variability, including the DSS phenotype. There is at least one t(X;Y) SRY+ case reported with duplication of the *NR0B1* gene, but only partial gonadal dysgenesis (Table 3). It has been suggested that the Xp breakpoint in this case may have disrupted the 5' regulatory

Table 3. Clinical summary of t(X;Y) cases.

Karyotype	Phenotype	Reference
46,X,der(Y)t(X;Y)(p21;p11.3)	Female external genitalia, developmental delay/mental retardation, hypotonia, dysmorphic facial features, psychomotor retardation, streak gonads, gonadoblastoma, autoimmune disease.	Ogata et al., 1992
46,X,der(Y)t(X;Y)(p21.3;q11.21) *	Ambiguous external genitalia, mental retardation, muscular hypotrophy, dysmorphic facial features, psychomotor retardation, hypotrophic intra-abdominal testes, growth retardation.	Bardoni et al., 1993
46,X,der(Y)t(X;Y)(p21.1;q11)	Female external genitalia, developmental delay/mental retardation, hypotonia dysmorphic facial features, no identifiable gonads by ultrasound, growth retardation.	Bajalica et al., 1995
46,X,der(Y)t(X;Y)(p21;q11)	Ambiguous external genitalia, developmental delay/mental retardation, hypotonia, dysmorphic facial features, psychomotor retardation, growth retardation (gonads not examined).	Vasquez et al., 1999
46,X,der(Y)t(X;Y)(p21.2;p11.3)	Female external genitalia, developmental delay/mental retardation, hypotonia, dysmorphic facial features, no identifiable gonads by ultrasound, growth retardation, partial agenesis of the corpus callosum.	Sanlaville et al., 2004
46,X,der(Y)t(X;Y)(p21.1;p11.3)	Female external genitalia, developmental delay/mental retardation, hypotonia, dysmorphic facial features, no identifiable gonads by ultrasound, slight growth retardation, partial agenesis of the corpus callosum.	Present case

*A case with confirmed *NR0B1* gene duplication but only with partial gonadal dysgenesis.

region of the *NR0B1* gene resulting in incomplete DSS (Barbaro et al., 2008). In contrast, the patient reported here has a breakpoint that is further centromeric, containing the entire *NR0B1* gene and control elements and therefore exhibiting complete DSS.

The risk of developing a gonadal malignancy for these patients is the same as those faced by other phenotypic females who carry Y-chromosome material. Therefore pre-emptive removal of the gonadal tissue is often recommended.

The X;Y translocated forms of Xp21 duplication are isolated events, which arise *de novo* during paternal meiosis. This is in contrast to patients with interstitial Xp21 duplications and X;A translocations, which can be inherited from phenotypically normal female carriers (Barbaro et al., 2007). These scenarios have significantly different genetic counselling implications with family studies a likely recommendation in the latter two instances.

Until recently, the diagnosis of cases with X;Y translocations was characterised using GTG-banding and FISH. The latter method requires multiple rounds of testing, which is time-consuming and expensive. In contrast, the combination of GTG-banding and CMA used here is more straight forward. Critically, neither conventional GTG-banding nor CMA alone could detect both the presence of the DSS locus and the translocation in the patient reported here. Therefore, together, these techniques aid in identifying chromosome rearrangements and dosage changes in genes that play a pivotal role in disorders of sex development.

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