Short reports

De novo der(X)t(X;10)(q26;q21) with features of distal trisomy 10q: case report of paternal origin identified by late replication with BrdU and the human androgen receptor assay (HAR)

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Abstract

We describe an 11 year old girl with a de novo unbalanced t(X;10) that resulted in a deletion of Xq26→Xqter and a trisomy of 10q21→10qter. Her clinical features were of distal trisomy 10q, but she lacked the cardiovascular and renal malformations observed in duplications of 10q24→10qter and had only moderate mental retardation. X inactivation was assessed on peripheral blood lymphocytes by late replication with BrdU (LR) and the human androgen receptor assay (HAR). By LR the der(X) was inactive without spreading to 10q21→10qter in all cells. The HAR assay showed skewed methylation of the paternal allele (90%). The correlation of HAR and LR suggests that the der(X) was paternally inherited and is consistent with data from other de novo balanced and unbalanced X;autosome translocations

detected in females. This is the first report of parental origin of a de novo trisomy 10q.

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Unbalanced rearrangements involving chromosomes X and 10 are a rare cause of partial trisomies of chromosome 10. We are aware of only one case of trisomy 10pter→10q25 and monosomy Xpter→Xq11 inherited from a maternal X;10 translocation.¹ This paper presents a new case of an unbalanced X;10 translocation that resulted in a de novo deletion of Xq26→Xqter and trisomy of 10q21→10qter in a girl with features of distal trisomy 10q. We also report for the first time the combined use of the androgen receptor assay (HAR) and the analysis of X inactivation by late replication with BrdU to identify the parental origin of a de novo distal trisomy 10q.

Case report

CLINICAL FINDINGS

An 11 year old white girl was referred for genetic evaluation because of developmental delay and craniofacial, chest, and limb dysmorphism. Her physical examination showed the following: short stature (111 cm, <5th centile, 50th centile for 5½ years), microcephaly (46.8 cm, <5th centile, 50th centile for 14 months) with a normal skull shape, small and posteriorly rotated ears, epicanthic folds, short, downward slanting palpebral fissures with ptosis (surgically repaired at 10 years), flat nasal bridge with upturned tip of the nose, malar hypoplasia, facial asymmetry with prominence of left side, narrow and high arched palate, crowded teeth and pointed chin, hypoplastic nipples, and chest prominence of the left side owing to thoracic scoliosis. On the extremities there were bilateral short 5th metacarpals with a short proximal phalanx of the 5th finger, proximally placed thumbs, 2-3 syndactyly of her toes, and small hands and feet.

Figure 1 GTG banded metaphase showing the der(X).

Figure 2 Ideogram of the unbalanced t(X;10). The arrows show the breakpoints on 10q21 and Xq26. Both chromosomes 10 are normal while the der(X) shows a deletion of Xq26→Xqter and the translocated segment 10q21→10qter.

Figure 3 The arrow shows the late replicating derivative X without spreading of inactivation to 10q21→10qter.

CYTOGENETIC STUDIES

Chromosomes from peripheral blood lymphocytes were analysed by GTG banding (500 bands). An unbalanced translocation X;10 that resulted in a duplication of 10q21.2→10qter and a deletion of Xq26→Xqter was observed (karyotype 46,X,der(X)t(X;10)(q26;q21.2)) (figs 1 and 2). Parental chromosome studies were normal.

X inactivation was studied in 100 lymphocyte metaphases by the late S phase labelling technique with BrdU developed by

Dutrillaux *et al.*² A preferential inactivation of the derivative X without spreading to 10q21→10qter was observed (fig 3).

No studies were done in fibroblasts because the parents would not consent to a skin biopsy.

ANDROGEN RECEPTOR X INACTIVATION ASSAY

The human androgen receptor assay (HAR) was used to study the methylation of *Hpa*II sites in the first exon of the androgen receptor locus because it correlates closely with X inactivation. This PCR based assay is highly informative (90% heterozygosity) and distinguishes paternal from maternal alleles at Xq11.2-q12 based on the methylation status of a CpG region adjacent to a polymorphic CAG repeat.³ This molecular analysis of X inactivation was correlated with the late replicating status of the abnormal X to identify its parental origin. The PCR primers described by Allen *et al.*⁵ were used to amplify the region containing the polymorphic trinucleotide repeat in the androgen receptor gene. The forward primer was synthesised with 5' amino link residue which was covalently bound to carboxy-fluorescein (Molecular Probes).⁴ DNA samples from the proband and her parents were amplified in a 12.5 µl reaction consisting of 10 mmol/l Tris-HCl, 50 mmol/l KCl, 1.5 mmol/l MgCl, dNTPs (200 nmol/l each), primers (175 ng each), and 1.25 U *Taq* polymerase (Perkin Elmer Cetus). PCR conditions were 95°C for five minutes followed by 28 cycles of 96°C for 45 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Five microlitres of the PCR product were added to 10 µl loading solution (95% formamide, 20 mmol/l EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol). Samples were heated at 95°C for two minutes and immediately placed on ice. Three microlitres were loaded onto a sequencing gel, electrophoresed, and analysed by an automated laser fluorescent DNA sequencer detection system (DuPont Genesis 2000 DNA analysis system). Using the unincorporated primers, the lanes were manually assigned with subsequent automatic lane tracking.⁵

To assess X inactivation, an aliquot of the patient's DNA was digested with 10 U *Hpa*II and 10 U *Cfo*I (Boehringer Mannheim) in a 25 µl reaction volume at 37°C for two hours. Following the digestion the sample was extracted with phenol/chloroform and ether to inactivate the restriction enzymes. Five microlitres of the digested DNA were amplified by PCR as previously described. The digested sample was loaded onto the sequencing gel adjacent to the undigested DNA samples amplified by PCR from the patient and both parents.⁵

The relative sizes of the PCR products, amplified from the androgen receptor gene alleles, as indicated by the major peaks, were visually assessed. Each parental X chromosome and the maternal and paternal alleles inherited by the proband were unequivocally identified. The father had a single peak (P1, panel D, fig 4). The patient and her mother had two peaks each (M1P1 in panel B and M1M2 in panel A, respectively; fig 4). The proband showed an extremely skewed methylation of

Figure 4 X inactivation patterns of the androgen receptor alleles in the proband and both parents measured by the HAR assay on peripheral blood leucocytes. Panel A shows the mother carries alleles M1M2. Panel B shows the proband has the M1P1 alleles, while panel C shows the methylation of the paternal allele P1 after a DNA digestion with HpaII and CfoI. In panel D the father shows one allele, P1.

the paternal allele P1 (90%) in DNA digested with HpaII and CfoI from blood leucocytes (panel C, fig 4). Since the der(X) is 100% late replicating in lymphocytes and 90% of the paternal HAR allele is methylated (inactive) in blood leucocytes, these data correlate and suggest a paternal origin of the der(X).

Discussion

We report an unbalanced de novo X;10 translocation that resulted in a deletion of Xq26→Xqter and a trisomy of 10q21→10qter in a girl with features of distal trisomy 10q (table 1). The molecular-cytogenetic analysis of X inactivation in blood cells showed that it was inherited from the father and is the first

Table 1 Comparison between distal trisomy 10q and present case

Distal trisomy 10q	Present case
dup 10q24→10qter	dup 10q21→10qter
Growth and performance	
Profound mental deficiency	Moderate mental retardation
Severe pre- and postnatal growth retardation	Moderate developmental delay
Craniofacial	
Microcephaly	Microcephaly
Microphthalmia	
Flat face with prominent forehead	
Blepharophimosis/blepharoptosis	Blepharophimosis/blepharoptosis
Broad/depressed nasal bridge	Flat nasal bridge/upturned nose
High arched/cleft palate	High arched palate
Malformed/posteriorly rotated ears	Small posteriorly rotated ears
Bow shaped mouth	
Prominent upper lip	Thin lips
Limbs	
Camptodactyly	
2-3 syndactyly of toes	2-3 syndactyly of toes
Proximally placed thumbs	Proximally placed thumbs
	Small hands and feet
	Slender limbs
Other	
Heart malformations	Scoliosis
Renal malformations	
Kyphoscoliosis	
General hyperlaxity	
Natural history	
50% death rate in 1st year of life owing to complications of the congenital malformations	At present 11 years old and having some resource help at school

Reviewed by Fryns et al. Clin Genet 1987; 32:61-5.

parental origin identification of a de novo distal trisomy 10q. Trisomy 10q is a distinct chromosomal syndrome characterised by growth retardation of prenatal onset and craniofacial, skeletal, and other somatic anomalies.^{6,7} There is usually severe mental retardation, but mild to moderate intellectual deficit, as in our proband, has been observed.⁶ Long term prognosis is usually poor owing to complications of heart and renal malformations (table 1). Most cases are unbalanced products from autosomal translocations and the X chromosome is rarely involved.⁸ To our knowledge there is only one previous publication of a child who inherited a der(X) from a maternal X;10 translocation. Our patient is the second report of a der(X)t(X;10), is “de novo”, and has craniofacial, skeletal, hand, and foot anomalies compatible with trisomy 10q24→10qter syndrome, but does not have cardiovascular or renal malformations which usually reduce the survival rate to 1/2 or 1/3 in the first year of life (table 1). Her dysmorphism resembles the duplications 10q25→10qter which have a milder phenotype than trisomy 10q24→10qter (normal or almost normal birth weight, most of the features of trisomy 10q24→10qter but with absence of heart, renal, or palatal malformations, and longer life span).^{6,9} This seems consistent with the view that the “critical region” for distal trisomy 10q syndrome is q24→qter and that bands q21→q24 did not contribute significantly to the phenotype in this case. Because of the proband’s age the endocrinological features documented in deletions of Xq26→Xqter could not be evaluated (normal

adult height, spontaneous pubertal development, and premature ovarian failure).¹⁰ We speculate that none of them may develop because of the skewed inactivation of the der(X) detected by our molecular-cytogenetic studies. It could be argued that this skewed inactivation is unrelated to her phenotype because such an event can be a mendelian trait in a few families¹¹ and approximately 10% of normal females have shown this pattern by the HAR assay.⁵ However, in this case we believe this is unlikely for the following reasons. (1) The late replication of the derivative X without spreading of inactivation to 10q21→10qter in lymphocytes is consistent with the phenotype of distal trisomy 10q. This X inactivation pattern is the most frequently seen in unbalanced X;autosome translocations.¹² (2) X inactivation would be initially non-random but afterwards the somatic cells with an inactive der(X) and a normal active X probably were at a selective advantage because selection mechanisms would favour the least deleterious chromosomal imbalance. (3) If the whole der(X) is active and the normal X is inactive the karyotype would have been more unbalanced and potentially lethal (monosomy of Xq26→Xqter and trisomy of 10q21→10qter). The paternal origin of this der(X)t(X;10) is consistent with observations in other de novo balanced and unbalanced X;autosome translocations in females.¹³⁻²² This suggests that the paternal X is prone to rearrangements through abnormal mechanisms of breakage and recombination. The unpaired regions of the X may synapse and recombine with autosomes during the initial stages of meiosis when heterologous pairing is observed¹⁶ or a postmeiotic non-homologous recombination could occur during spermiogenesis since X;autosome translocations usually lack transmission through male meiosis.¹⁴

Finally, this report and studies of another unbalanced (X;3) translocation²³ show that the methylation of the androgen receptor (HAR) alleles at Xq11.2-q12 correlates with the late replication status of the der(X). Both cases were de novo and late replicating and the paternal HAR allele was methylated so their paternal origin was inferred. We conclude this is a simple, fast, and reliable molecular-cytogenetic approach to identify parental origin that at the same time permits the analysis of X inactivation.

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