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Case report

A familial X/Y translocation: cytogenetic and molecular study

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Abstract. In this study we describe a 3-generation family carrying a (X;Y)(p22.3;q11.2) translocation in seven individuals of both sexes. Molecular analysis of the aberrant (X;Y)(p22.3;q11.2) chromosome was performed by FISH using X and Y-specific painting probes and also PCR amplification of the Y-specific sequences. Using these approaches it was demonstrated that the translocation resulted in a deletion of both X and Y pseudoautosomal regions. Moreover, using RBG banding it was shown that in all females the X-derivative chromosome was inactive in over 90% of mitoses. From the preliminary results obtained in this study we assumed that in this particular family the observed phenotype of the patients was caused by a deletion of the cluster of pseudoaotosomal genes responsible for the stature. More proximal *loci,* like STS or MRX49, were probably not deleted, since neither ichtyosis nor mental retardation was observed in this family.

Key words: hirsutism, X/Y translocations.

During male meiosis highly homologous regions on Xp22.3 and Yq11.2 can cause the X and Y chromosomes to undergo abnormal pairing and exchange, leading to (X;Y)(p22.3;q11.2) translocations. This event results in a portion of the distal part of Yq being translocated to Xp and a terminal Xp22.3 deletion (BALLABIO et al. 1989). This type of rearrangement is the most frequent among X/Y translocations (HSU 1994). The common phenotypic feature related to this chromosomal aberration is short stature because of haploinsuficiency of the pseudoautosomal *SHOX* (short stature homeo box) gene (RAO et al. 1997). However, other characteristics of affected individuals depend on gender and the location of the breakpoints on

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the X and Y chromosomes. For instance, in male patients the following recessive diseases can be present: a mild type of X-linked chondrodysplasia punctata (CDPX1) due to nullisomy of the *ARSE* (arylsulphatase E) gene, mental retardation due to nullisomy of a hypothetical *MRX* (mental retardation X-linked) gene, X-linked ichthyosis due to nullisomy of the *ARSC1* (arylsulphatase C1) gene, and Kallman syndrome due to nullisomy of the *KAL1* gene.

In this study, we performed molecular and cytogenetic analyses of a family with (X;Y)(p22.3;q11.2) translocation in order to correlate the phenotype present in this family with the chromosomal rearrangement.

The family studied was of Polish origin, with seven affected individuals in three generations (Figure 1). The proband (III-2), a 4-year-old girl, was evaluated because of short stature and skeletal dysplasia characterised by a slightly disproportional stature with short limbs. Her height was 93 cm (-3 SD) and weight was 15 kg (3-10 centile). She had a retarded bone age, a depressed nasal bridge, bilateral simian creases, and a broad chest. The brother of the proband (III-1) at 3 years

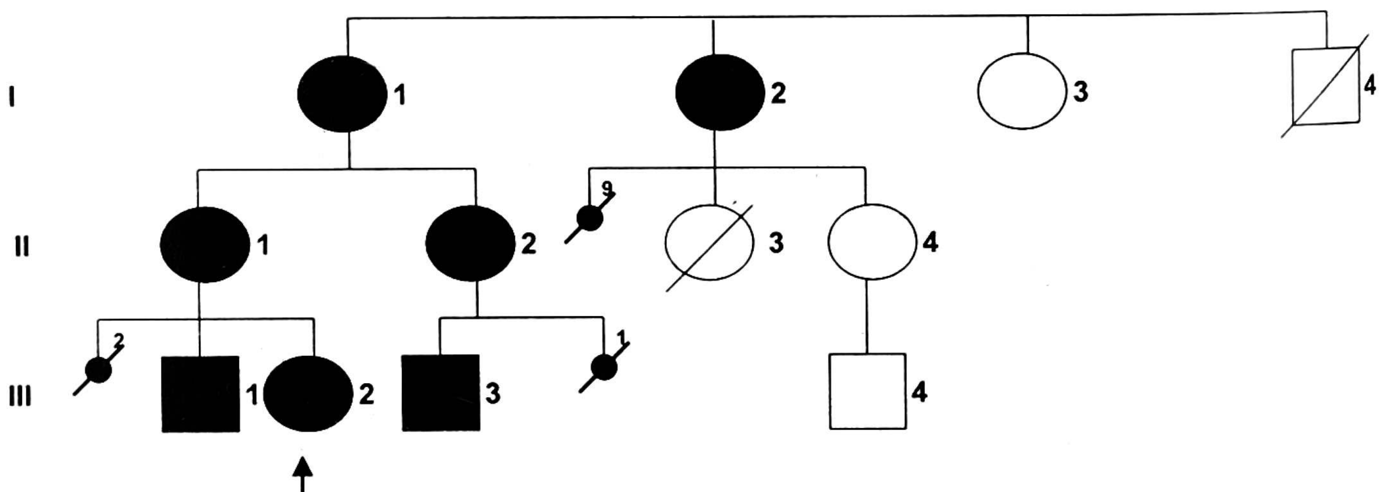
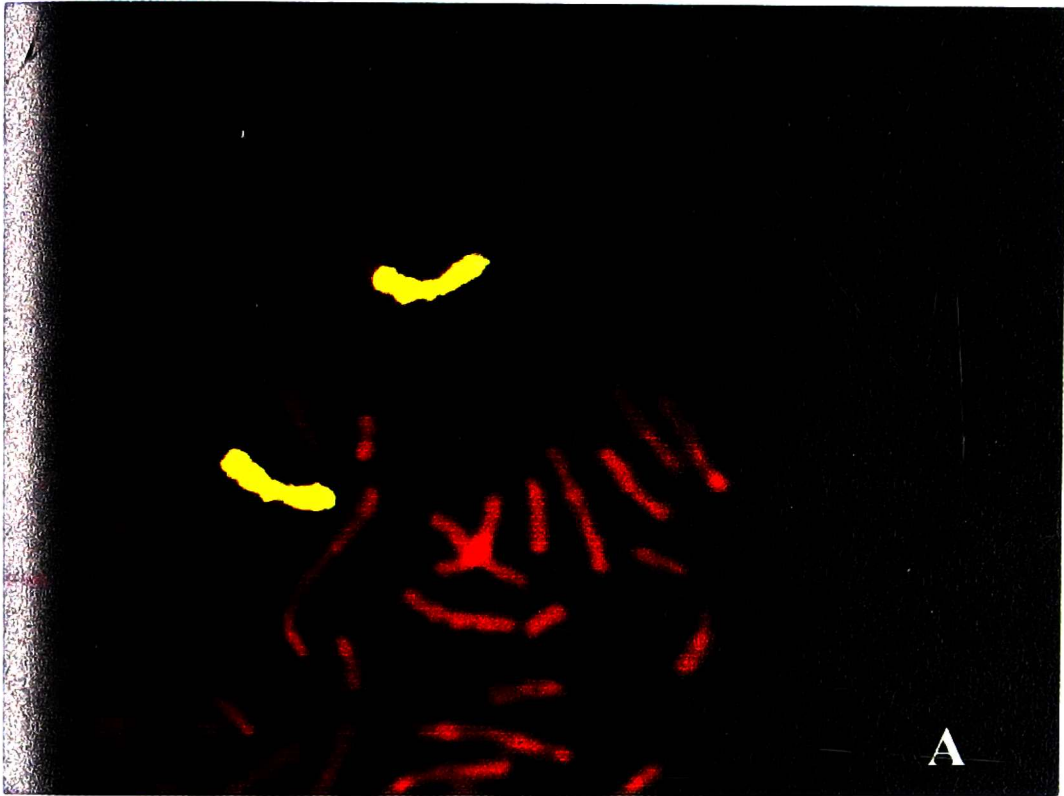


Figure 1. Pedigree of the family. The proband is marked with an arrow. Roman numerals assigning to generations, Arabic numerals to sequential individuals at each generation. Small numerals assigning to number of miscarriages.

of age was 87 cm tall (-3 SD) and his weight was 12 kg (-3 SD). He had a skeletal displasia similar to that of the proband, a broad nasal bridge, bilateral simian creases, and a broad chest. The mother of the proband (II-1) was 150 cm tall, had polycystic ovaries, hirsutism and retroflexion of the uterus. In addition to the two affected children, she had had two miscarriages. The cousin of the proband (III-3) was 2.4 kg at birth. The aunt of the proband (II-2) was 145 cm tall, had irregular menses, an increased level of testosterone and prolactin, and hirsutism. In addition to the one affected child, she had had one miscarriage. The grandmother of the proband (I-1) was 145 cm tall and had hirsutism. The grandmother's sister (I-2) was 147 cm tall and had hirsutism. In addition to one healthy daughter and one daughter who died of leukemia at two years, she had had 9 miscarriages.



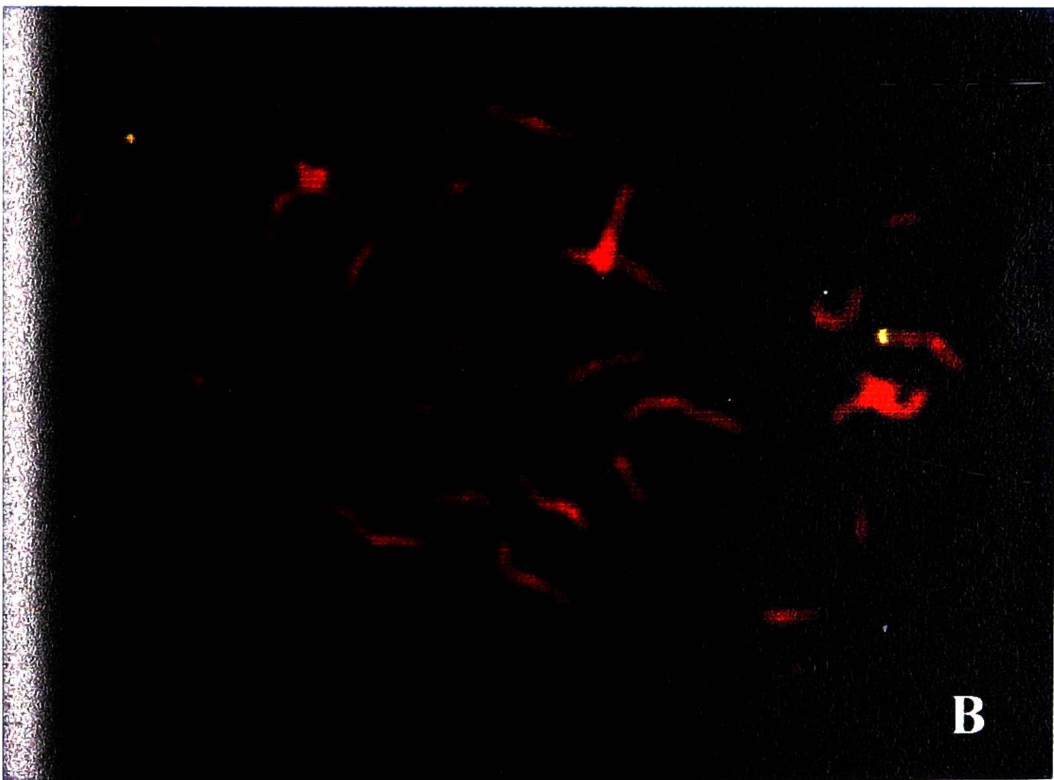


Figure 2 A. FISH with a painting probe for the X chromosome in a female patient showing that the rearranged chromosome is an X derivative. B. FISH with a painting probe for the Y chromosome in a male patient, showing the presence of the Y chromosome fragment on an X derivative.

We performed a cytogenetic and molecular analysis in one unaffected (II-4) and all the affected (III-2 III-1, II-1, III-3, II-2, I-1 and I-2) members of this family. Chromosome HRBT studies were carried out on peripheral blood lymphocytes using GTG, RBG, CBG and QFQ banding techniques. The karyotype of each patient was analysed in at least 100 metaphases. Discrimination between the active and inactive X-chromosomes was done by RBG banding of metaphase chromosomes. For a more precise analysis of the aberrant chromosome, fluorescent *in situ* hybridisation (FISH) was performed using X and Y-chromosome painting probes labelled with digoxigenin. Amplification and detection of hybridisation signals were done by immunocytochemical reaction followed by analysis using a fluorescence microscope equipped with a CCD video camera and Meta-system image analyser.

For molecular evaluation of the Y-chromosome content of aberrant chromosomes, genomic DNA of affected females was isolated from the peripheral blood lymphocytes by standard techniques (SAMBROOK et al. 1989). DNA amplification was performed using polymerase chain reaction (PCR) and primers complementary to Y specific sequence tagged sites (STSs) corresponding to intervals 5, 6 or 7 of the Yq (annealing temperatures are given in parentheses): sY79 (52°C), sY84 (60°C), sY87 (55°C), sY90 (55°C), sY95 (60°C), sY593 (58°C), sY595 (58°C), sY638 (58°C), sY591 (58°C), sY202 (58°C), and sY159 (58°C) according to VORLATH et al. (1992), FOOTE et al. (1992). Amplification reactions were performed in 20 μl volume, containing 50 mM KCl, 10 mM Tris-HCl, pH 8.4, 5 mM $MgCl_2$, 0.15 mM each dNTP, 0.5 μM each amplification primer and 1 U of Taq polymerase. The conditions were as follows: 94°C for 5 min, then 30 cycles at 94°C for 30 s, annealing for 30 s at the appropriate temperatures, 72°C for 45 s and 72°C for 7 min.

By classical cytogenetic lymphocyte analysis an X chromosome translocation to other chromosomal material causing an Xp22.3 deletion was identified in III-2, III-1, II-1, III-3, II-2, I-1 and I-2. The use of FISH with painting probes specific to X and Y chromosomes enabled us to find that both chromosomes were involved in the translocation (X;Y)(p22.3;q11.2). The following karyotypes were identified: non mosaic karyotype 46,Y,der(X)t(Xp22.3;Yq11.2) in 2 boys (III-1 and III-3), and mosaic 45,X/46,X,der(X)t(Xp22.3;Yq11.2)/47,X,der(X)t(Xp22.3;Yq11.2), der(X)t(Xp22.3Yq11.2) in 5 women (III-2, II-1, II-2, I-1 and I-2). Moreover, the cell line carrying the 46,X,der(X)t(Xp22.3;Yq11.2) translocation was predominant in each female patient, the derivative X chromosome being the late replicating one in about 90% of the analysed mitoses. The unaffected woman (II-4) had a normal karyotype.

Results of PCR analysis of the 11 Y-specific STS sequences in female patients carrying X/Y translocations are presented in Table 1. In each case we detected the same portion of the Y chromosome containing STSs specific for intervals 5L, 6 and 7, confirming the cytogenetic data that the translocated portion was Yq and situating the breakpoint between sY95 (interval 5H) and sY638 (interval 5L).

The affected members of this family had short statures typical for this type of X/Y translocations: in III-2, II-1, II-2, I-1 and I-2 females and in III-1 and III-3 males. However, no ichtyosis or mental retardation were observed. These features are present when the deletion encompasses more proximal loci, such as steroide sulphatase (STS) or MRX49 *locus,* respectively (VALDES-FLORES et al. 2000, SPRANGER et al. 1999).

Interestingly, all adult female patients (II-1, II-2, I-1 and I-2) in this family show hirsutism, which has not been described in X/Y translocations to date, to our knowledge. Hirsutism is known to frequently coexist with polycystic ovaries (PCO) and elevated level of androgens (YEN, JAFFE 1991). Since one of our patients (II-1) was diagnosed with the PCO and another (II-2) had an increased level of testosterone, we conclude that hirsutism in this family was related to PCO phenotype. Further molecular studies of the X/Y translocation are needed for a better understanding of the expression of X-linked recessive CDPX in the one female.

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