

SHORT COMMUNICATION:

Clinical and molecular studies in a mother and son with Xp22.3 deletion

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ABSTRACT

We studied a young man presenting short stature, moderate mental retardation, seizures, ichthyosis, anosmia and hypogonadotrophic hypogonadism (Kallmann's syndrome). The cytogenetic analysis identified a maternally inherited X-Y translocation (Xp22.3;Yq11). The results of the molecular studies were compatible with contiguous gene syndrome. The presence of decreased carpal arch in the mother and deformities of the medial tibial condyle in both the propositus and his mother associates these abnormalities with the loss of a segment of the X chromosome.

INTRODUCTION

Contiguous gene syndrome is the phenotypic expression of several Mendelian diseases in one patient due to a deletion or duplication of a segment of DNA, sometimes visible with cytogenetic techniques. One of these syndromes is caused by a deletion of the Xp22.3 → Xpter region (Ballabio *et al.*, 1989).

The complete picture of the contiguous gene syndrome of the Xp22.3 region includes: short stature (MIM312865), X-linked chondrodysplasia punctata (MIM302950), mental retardation (MIM309530), X-linked ichthyosis (MIM308100) and Kallmann's syndrome (MIM308700). The assignment of these genes was made by genotype-phenotype correlation in

patients with interstitial and terminal deletions restricted to the Xp22.3 region.

Zuffardi *et al.* (1982) observed isolated short stature associated with terminal deletions of the X chromosome. Males with Xp22.3 → Yq11 translocations, nulissomic for the distal segment of the X short arm, are examples of patients having associated short stature and chondrodysplasia punctata (Ballabio *et al.*, 1989; Van Maldergen *et al.*, 1991), and a more extended phenotype with mental retardation and X-linked ichthyosis (Tiepolo *et al.*, 1980; Akesson *et al.*, 1980; Agematsu *et al.*, 1988; Ballabio *et al.*, 1989; Matsumoto *et al.*, 1991). Simple terminal deletion of the Xp22.3 region was also described in one family in which the deleted males were affected by short stature, X-linked chondrodysplasia punctata, mental retardation, and X-linked ichthyosis (Curry *et al.*, 1984). Association of X-linked ichthyosis and Kallmann's syndrome was observed in some males of one family, suggesting a close linkage between the two conditions (Ballabio *et al.*, 1986). Further examination of the patient with X-Y translocation described by Tiepolo *et al.* (1980) also

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confirmed the association with Kallmann's syndrome. The heterogeneous phenotypes observed among those patients suggested differences at a molecular level. The ordering of the loci of the diseases associated with the Xp22.3 region was made by Ballabio *et al.* (1989) using deletion mapping in some of the above patients, plus some others with similar clinical features and X terminal deletions. Studies of linkage analysis in families with isolated Kallmann's syndrome also mapped its locus at Xp22.3 (Meitinger *et al.*, 1990).

X-linked ichthyosis was first assigned to Xp in cases of X-autosomal translocation and the steroid sulfatase gene was the first cloned from the Xp22.3 region (Ballabio *et al.*, 1986; Yen *et al.*, 1986). The Kallmann's gene was cloned by Franco *et al.* (1991) who named the gene KALIG-1, and Legouis *et al.* (1991), who termed the gene ADMLX (adhesion molecule-like from the X-chromosome). The other genes have not been cloned yet. The deletion of the KALIG-1 gene was previously demonstrated in the DNA of our proband by Franco *et al.* (1991).

Figure 1 shows the relative position of the conditions related to the contiguous gene syndromes of the Xp22.3 region (as published by Ballabio *et al.*, 1989) and the DNA sequences used in this study.

SUBJECTS, METHODS AND RESULTS

Proband

MHBS (Figure 2) was born from noncon-sanguineous young parents after a full-term twin pregnancy by normal delivery. His twin brother weighed 2,800 g and died on his third day of life of unknown cause. The proband's birthweight was 1,880 g. He was a small infant with failure to thrive. His skin was extremely dry and scaly and, by the age of one year, a skin biopsy diagnosed ichthyosis. His psychomotor development was slightly retarded. By the age of two he presented seizures and has been on anticonvulsant therapy since then. He has attended a specialized school for learning disabilities. He was referred to our endocrine and genetic department at the age of 13 because of moderate mental retardation, ichthyosis, short stature and small penis. Hormonal assays revealed very low levels of testosterone and the LHRH stimulation test was negative. The X-ray studies at 14 years of age demonstrated short 4th metacarpals and normal carpal angles. The radiographs of the knees showed epiphysis and metaphysis still not fused with

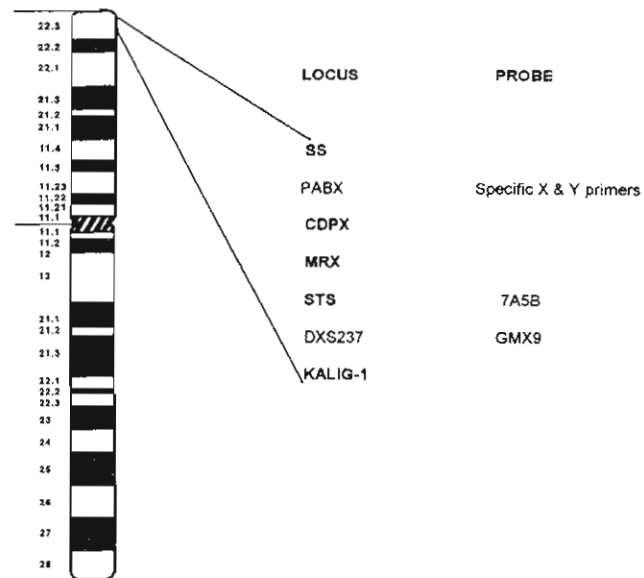


Figure 1 - Schematic representation of the relative locations of the diseases of the contiguous gene syndrome of the Xp22.3 region and the DNA sequences used in this study (The order of the diseases is presented as described by Ballabio *et al.*, 1989). SS = Short stature; CDPX = X-linked chondrodysplasia punctata; MRX = X-linked nonspecific mental retardation; STS = steroid sulfatase locus deleted in X-linked ichthyosis; KALIG-1 = gene of the Kallmann's syndrome.

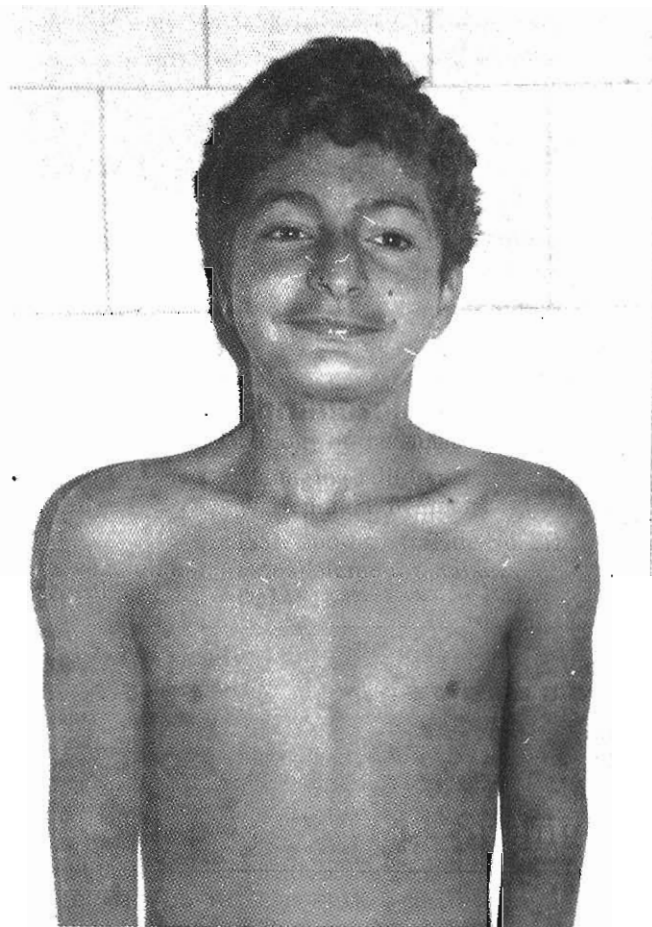


Figure 2 - Photograph of the proband at the age of 14.

pointed projections of the medial portion of the tibial epiphysis. The medial condyle was lower than normal and the medial metaphysis was already beaked downward (Figure 3). Anosmia was subsequently diagnosed. By the age of 14 he started testosterone replacement, with appropriate virilization. Recent physical examination at 24 years of age revealed a pleasant, moderately retarded man with very short stature (142 cm), hypoplastic alae nasi, malar hypoplasia, bilateral campto and clinodactyly of the 5th finger, severe and generalized ichthyosis, male genitalia with small penis and hypoplastic scrotum. He is still dependent on anticonvulsant drugs.

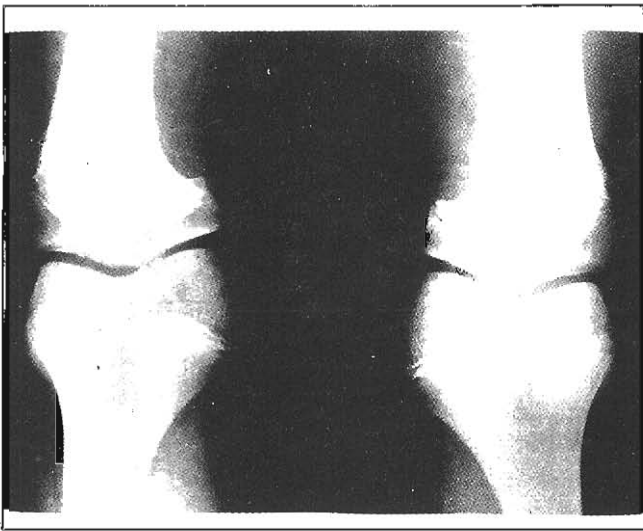


Figure 3 - Radiographs of the knees of the proband at the age of 14.

Mother

MHB was first examined when she was 39 years old. She was born from nonconsanguineous parents and had a normal brother and sister. Edema of the extremities at birth was not recalled by her mother. Her previous history revealed slow growth since infancy and normal intelligence, with good performance at school. She presented telarche at 10 years of age, menarche occurred about one year later and she always had regular menses. She was 23 years old at the delivery of the proband. Subsequently she had one pregnancy loss at first trimester and two pregnancy losses at second trimester. No abnormalities of the fetuses were recorded. Physical examination showed an intelligent woman, 140 cm in height and 56 kg in weight, with bilateral shortening of the 4th metacarpals. No other abnormalities were found and the gynecological examination was normal. The radiographs of the hands and wrists showed bilaterally reduced proximal carpal

angle of 115° , compared with the mean normal value of 131° , described by Kosowicz (1962). The radiographs of the knees showed the following symmetrical findings: medial femoral condyle lower than normal with depression of the medial plateau and downward beaked projection of the medial tibial condyle.

Cytogenetic studies

Cytogenetic investigation in R,G,C banded and G-11 stained preparations showed a 46,Y,der mat(X)(p22.3;q11) in the proband (Figure 4). His mother's karyotype was 46,X,t(X;Y) (p22.3;q11). The maternal grandparents had normal chromosomes. We studied the chromosomal replication using 5-BrDU in blood cultures of the proband's mother. Analysis with C bands showed preferential inactivation of the abnormal X in 85% of the cells. In a considerable number of them we observed the X inactivation spreading effect to the Yq material.

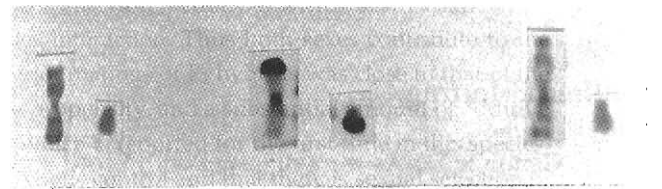


Figure 4 - X-Y translocation and normal Y chromosomes of the proband with G, C and R bands, respectively.

Molecular studies

The molecular studies were only performed in the proband to analyze the segment deleted in the X-chromosome. DNA from normal and fertile men and women were used as controls.

High molecular weight DNA was extracted from the blood using the method described by Kunkel *et al.* (1977).

PCR

Primers specific for X and Y chromosomal sequences and a primer for the pseudoautosomal region were provided by Dr. Peter Goodfellow. Amplification was performed as previously described (Pereira *et al.*, 1991).

The amplification products showed absence of the 0.95 kb specific X band of PABX locus in the proband. The 1.1 kb specific Y band of the PABY locus was the product of the normal Y (Figure 5).

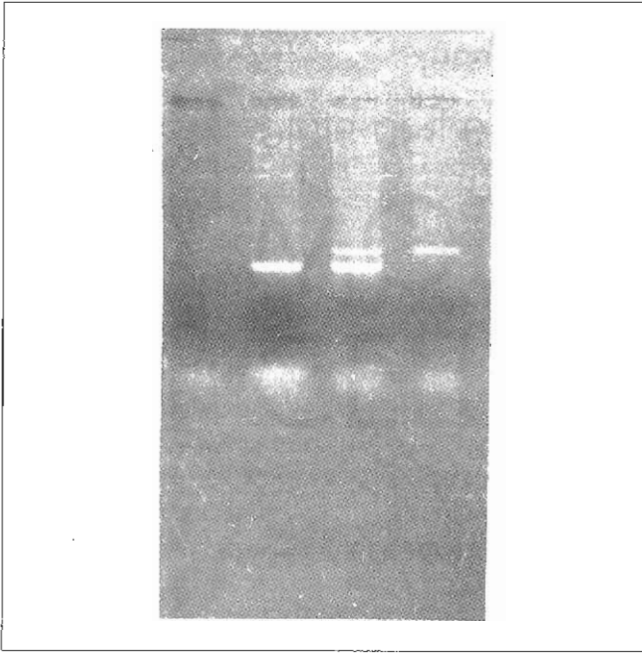


Figure 5 - PCR amplification with primers specific for the X and Y region of the pseudoautosomal boundaries. Track [1] negative control, [2] normal man, [3] normal woman, [4] proband (deleted the boundary region of the X chromosome).

Southern blotting

Genomic DNAs were digested with EcoRI and HindIII and analyzed with the probes 7A5B and GMGX9, respectively. The 7A5B probe was kindly provided by Dr. Andrea Ballabio and the GMGX9 probe was obtained from the European Cell Bank. Restriction enzymes were used as recommended by the manufacturer (Anglian). Migration was made in 1% agarose with the same TBE buffer used for electrophoresis. DNA was transferred to Hybond N membranes (Amersham) using Southern's method (Southern, 1975). Prehybridization and hybridization solution were the same with 0.5 mol/l sodium phosphate/7% SDS pH 7.0 with 200 µg/ml denatured salmon sperm DNA. These procedures were performed at 65°C in a Bachoffer hybridization oven. Prehybridization was for three hours and hybridization overnight. Probe inserts were labelled in a random hexanucleotide labelling reaction (Amersham) with ³²P-dCTP. Autoradiographs were developed overnight using Amersham Hyperfilm M.P.

The normal pattern of bands for the 7A5B probe was obtained in the controls and were absent in our patient, demonstrating deletion of the gene for steroid sulfatase recognized by that probe (Figure 6). The GMGX9 probe recognizes a more proximal segment of the X chromosome and was deleted in our patient and present in controls (Figure 7).

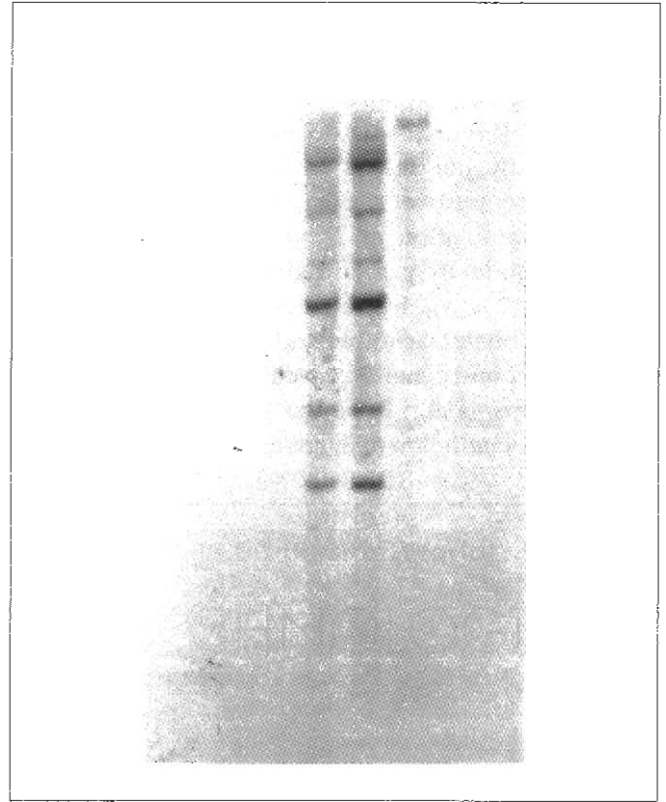


Figure 6 - Southern blot of EcoRI digests probed with 7A5B (detects the STS gene). Track [1] normal man, [2] normal woman, [3] proband (deleted segment).

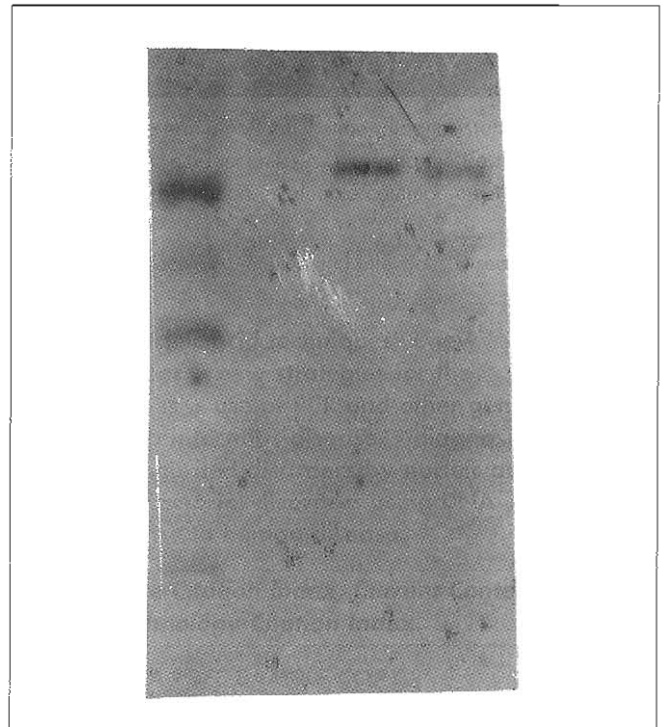


Figure 7 - Southern blot of HindIII digests probed with GMGX9 (detects locus DXS237, proximal to the STS gene). The probe can recognize one DNA fragment of 4 kb or DNA fragments of 2.5 kb and 1.5 kb if there is a restriction site for the enzyme used. Track [1] normal female (heterozygote), [2] proband (deleted segment), [3] and [4] normal male (homozygous).

DISCUSSION

The clinical picture of the proband represents the deletion of several genes of the Xp22.3 region: short stature, mental retardation, X-linked ichthyosis and Kallmann's syndrome.

The X-linked chondrodysplasia punctata was not detected by the mother in infancy or early childhood. The only X-ray picture we could find was performed at four years of age and it did not show the punctate calcifications in the epiphysis or the hypoplasia of the distal phalanges, described as distinctive in this disease (Curry *et al.*, 1982). Nevertheless, our patient presented the facial dysmorphism described in that condition. Intrafamilial variation of hypoplasia of the distal phalanges has already been described (Curry *et al.*, 1984).

The proband and his mother presented short stature, which is widely known for the Xp deletion. All female carriers of the Xp22.3-Y11 translocation described so far were short and fertile (Khudr *et al.*, 1973; Van den Berghe *et al.*, 1977; Tiepolo *et al.*, 1977; Akesson *et al.*, 1980; Pfeiffer, 1980; Agematsu *et al.*, 1988; Ballabio *et al.*, 1988; Van Maldergen *et al.*, 1991; Matsumoto *et al.*, 1991). The locus for short stature is located in the pseudoautosomal region of the X chromosome (Ballabio *et al.*, 1989). Its absence is always deleterious in female patients and expressed as failure of linear growth. The angular shape of the proximal carpal row observed in the proband's mother is the same as for Madelung's deformity and is recognized in patients with Turner's syndrome (Simpson, 1975). As demonstrated by Kosowicz (1962), the proximal carpal angle is in the normal range in other forms of short stature due to hormonal or chondrodysplastic abnormalities. The entire Madelung's deformity, as described in dischondroosteoses, namely a bayonet-like jog and distal protusion of the ulna was stated as absent in Turner's syndrome (Kosowicz, 1962), as it was in our patient. One patient with an X-Y translocation was described with evidence of mild dyschondrosteosis (Pfeiffer, 1980) and another with abnormal carpal angle (Van Maldergem *et al.*, 1991). Hsu *et al.* (1994) recently described Madelung's deformity in a mother and daughter who presented subtle deletion of the Xp22.31 band.

Deformity of the medial tibial condyle is well known in Turner's syndrome (Kosowicz and Poland, 1960; Simpson, 1975). The presence of this in our patients suggests that this phenotypic finding is associated with the Xp22.3 → pter segment. We did not find another description of this abnormality in other

male or female patients with Xp22.3 deletion, probably because it was not investigated.

The deletion in our patient involved the STS gene (Figure 4) and the proximal segment recognized by the GMGX9 probe (Figure 5). The deletion of the KALIG-1 gene was previously demonstrated in the DNA of our proband by Franco *et al.* (1991).

Isolated cases of X-linked ichthyosis and X-linked Kallmann's syndrome have demonstrated different distributions in relation to gene abnormality. While the former show deletion of the entire gene in 90% of the patients (Bonifas *et al.*, 1987; Shapiro *et al.*, 1989), in the latter only a minority of the patients showed deletions (Hardelin *et al.*, 1993), suggesting point deletions as the major defect.

In conclusion we consider that our patient had the entire phenotype of the Xp22.3 contiguous gene syndrome and that the reduced carpal angle and the Kosowicz sign of the knees are part of this phenotype.

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RESUMO

Estudamos um paciente que apresentava baixa estatura, retardo mental moderado, convulsões, ictiose, anosmia e hipogonadismo hipogonadotrófico (Síndrome de Kallmann). A análise citogenética identificou a translocação X;Y (Xp22.3;Yq11) de origem materna. Os resultados do estudo molecular foram compatíveis com uma síndrome de genes contíguos. A diminuição do ângulo carpal na mãe e as deformidades do côndilo medial da tibia em ambos relacionam essas anormalidades ao segmento cromossômico perdido do cromossomo X.

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