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Case report

Normal postnatal outcome in an r(X) mosaic male fetus with retained *XIST* gene

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Abstract

We report the case of a pregnant woman who underwent prenatal diagnosis by chorionic villi sampling for increased risk of trisomy 21 due to advanced age and abnormal results of the first trimester combined screening test.

Karyotype analysis of the chorionic villi sampling showed a normal male karyotype (46,XY) in 16 metaphases derived from the trophoblast culture and a mosaic in the mesenchymal culture for the presence of a supernumerary marker chromosome (SMC) in 6 metaphases (47,XY,+mar[6]/46,XY[16]).

To evaluate the presence of a real mosaicism, karyotype analysis was repeated on amniocytes derived from a single primary culture, confirming the presence of an abnormal cell line with a mosaicism of 27% (47,XY+mar[4]/46,XY[11]).

The distribution and the extent of the mosaicism were better characterized by the analysis of fetal blood, which allowed the definition of the SMC as an X derivative with a ring structure present at mosaic in 24% of the peripheral lymphocytes (47,XY,r(X)[23]/46XY[73]).

CGH-array on fetal blood-derived DNA defined the extent for 43 Mb of the X chromosome duplication from Xp21.1 to Xq21.1.

FISH analysis, using X centromeric and *XIST* probes, confirmed the X derivation of the marker and the inclusion of the *XIST* gene within the duplicated fragment.

Ultrasound fetal evaluation was unremarkable and the woman, counseled positively for the conservation of the *XIST* gene, decided to continue the pregnancy, proceeding to term.

The woman delivered an apparently normal male baby who, at the follow-up to 13 months, appears morphologically and developmentally normal.

Keywords

XIST gene, X-inactivation, SMC, r(X), mosaicism, prenatal diagnosis.

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Introduction

The presence of extra-numerary chromosomal fragments causing aneuploidy is usually due to structural chromosome rearrangement inherited from parents in 30% of the cases, whereas more frequently (70%) is an acquired anomaly occurred at the post-zygote stage [1, 2].

In familiar cases, the transmitting parent is generally the mother, since in male the supernumerary marker chromosome (SMC) may determine infertility or affect sperm viability, selecting against transmission of the anomaly to the embryo.

Rings of the X chromosome are rare cytogenetic anomalies occurring mainly in females in the context of a mosaic Turner syndrome (45,X/46,Xr(X)) with mild impact on the classical Turner phenotype [3, 4].

However, in patients with a small r(X), the phenotype is instead associated with intellectual disability and additional dysmorphic features that include facial dysmorphism, syndactyly, microcephaly, cardiac and skeletal anomalies [5].

This abnormal phenotype is caused by the loss of the *XIST* gene and the lack of X-inactivation, which leads to the expression in double dosage of normally repressed allelic X-genes.

Hence, the phenotype of the 47,XY,+r(X) is paradoxically more severe than the Klinefelter phenotype (usually 47,XXY), in which the supernumerary X-chromosome, even though entire, is generally almost completely silenced [6-8].

In males, r(X) are extremely rare, but often highly detrimental. The most important variable determining the evolution of the clinical phenotype is the inclusion or the exclusion of the *XIST* gene within the residual chromosomal fragment of the ring. The product of the *XIST* gene is a long RNA that initiates and maintains the inactivation of the X chromosome in cis [9].

Hence, the absence of the *XIST* gene is expected to impair the inactivation of the r(X), resulting in abnormal phenotypes. In fact, in all reports of the literature but one, r(X)s lacking the *XIST* gene in males result in the absence of X inactivation and pathological neonatal outcomes [10-13].

The absence of previously reported males born with the supernumerary r(X) bearing the *XIST* gene does not allow a reliable prediction of a favorable phenotype based on empirical evidence.

This is even more true in cases, like the one we describe, in which the supernumerary marker is not inherited, but occurs *de novo*, a condition that often carries a risk of imbalanced chromatin [14].

In this paper, we present the first case of a male with a *de novo* mosaic r(X) chromosome that includes the *XIST* gene and has an apparently normal male phenotype.

Hence, our patient's apparently normal phenotype at the one-year follow-up provides empiric support to the theory that the presence of the *XIST* gene in the ring should ensure a normal development.

Materials and methods

Karyotype analysis

Prenatal samples were karyotyped according to standard procedures for chorionic villi or amniotic fluid.

Cells from long-term cultured villi (LTC-villi) were treated with pronase and collagenase at 37° for 15 minutes, respectively.

Cells were cultured in Nunc chambers slides and harvested *in situ* after 7-9 days.

Amniocytes were treated according to Euroclone protocol, cultured in Nunc chambers slides and harvested *in situ* after 8-10 days.

Postnatal karyotype was performed using metaphase from phytohaemagglutinin-stimulated blood lymphocytes.

The metaphases were stained using the conventional QFQ-banding technique.

Chromosome analysis was done under fluorescence microscope with 100× magnification.

The analysis and nomenclature of the chromosome were based on ISCN 2016.

FISH analysis

FISH analysis was conducted using standard procedures according to the probe manufacturer (Cytocell®, Oxford Gene Technology).

Green control probe hybridized to X centromeric region, while red *XIST* probe hybridized to Xq13.2 loci. Images were captured using Genikon® Imaging Software (Nikon Corporation).

CGH array analysis

DNA labeling and hybridization were performed by using the Agilent® Oligonucleotide Array-Based CGH for Genomic DNA Analysis protocol (V7.3, 2014).

Labeled Test (Cy5) and Reference (Cy3) DNA samples were paired and co-hybridized to the SurePrint G3 Human CGH Microarrays, 4×44 K (Agilent®) at 67°C, 20 rpm for 24 h, then washed at room temperature by using the Agilent® Oligonucleotide Array-Based CGH for Genomic DNA Analysis protocol (V7.3, 2014).

The hybridized array was immediately scanned with an Agilent® Microarray Scanner (Agilent® Technologies, Inc.).

Authorization

We informed the parents of the intention to publish the description of their child anomaly. They comprehended the importance of the findings in assisting other prospective parents in the reproductive decision and they kindly authorized the publishing of the manuscript.

Results

Familiar anamnesis

A 39-year-old woman in the 12th week of pregnancy received genetic counseling for advanced age in our Hospital.

The familiar anamnesis reported a case of pervasive developmental disorder with intellectual disability in the sister's son and a case of epilepsy in a maternal cousin.

In the suspect of the fragile X syndrome, given the unavailability of the affected nephew, we investigated the mother as a potential carrier of the *FMR1* gene premutation [15].

Molecular analysis to evaluate the presence of premutation-mutation in the *FMR1* was normal; however, the subsequent first-trimester combined screening for aneuploidy suggested a high risk for trisomy 21, leading to the indication for invasive prenatal diagnosis and to fetal karyotype analysis on harvested chorionic villi.

Cytogenetic and molecular karyotype analysis

The result of chorionic villi sampling showed a discordance between the normal male karyotype found in the metaphases of the short-term trophoblast culture (46,XY[16]) and the abnormal karyotype found in the long-term mesenchymal culture, which showed a mosaicism with two cell lines: a line with normal male karyotype and a second line with an SMC (6 metaphases) not definable by classical cytogenetics banding.

To exclude the possibility of a placental confined mosaicism, we repeated the karyotype analysis on amniotic fluid at the 16th week of gestation, which confirmed the presence of two cell lines in one primary culture with a mosaicism around 27% (4 clones with SMC and 11 clones with normal male karyotype).

To further define the marker and the mosaicism distribution, fetal blood was harvested by cordocentesis at 20 weeks of gestation [16] and analyzed more extensively.

Karyotype derived from fetal lymphocytes reported a 24% of mosaicism (23 cells with SMC out of 96 total screened).

The better resolution of the banding on fetal blood allowed the characterization of the SMC as a small ring chromosome (**Fig. 1A**).

By FISH analysis using X-centromere specific probe, we determined the SMC derivation from the X chromosome, r(X) (**Fig. 1B**).

CGH array of fetal blood-derived DNA detected a duplication of the X chromosome extending for 43 Mb from Xp21.1 to Xq21.1: arr[GRCH37] Xp21.1p11.1(35253219_58324785)x2,Xq11.1q21.1(61932020_81631063)x2 (**Fig. 2**).

Karyotype analysis of both parents was normal (46,XX and 46,XY), proving the *de novo* origin of the SMC.

FISH analysis with X centromeric probe and an *XIST* specific probe confirmed the duplication of the X chromosome and of the *XIST* gene (**Fig. 1B** and **Fig. 1C**).

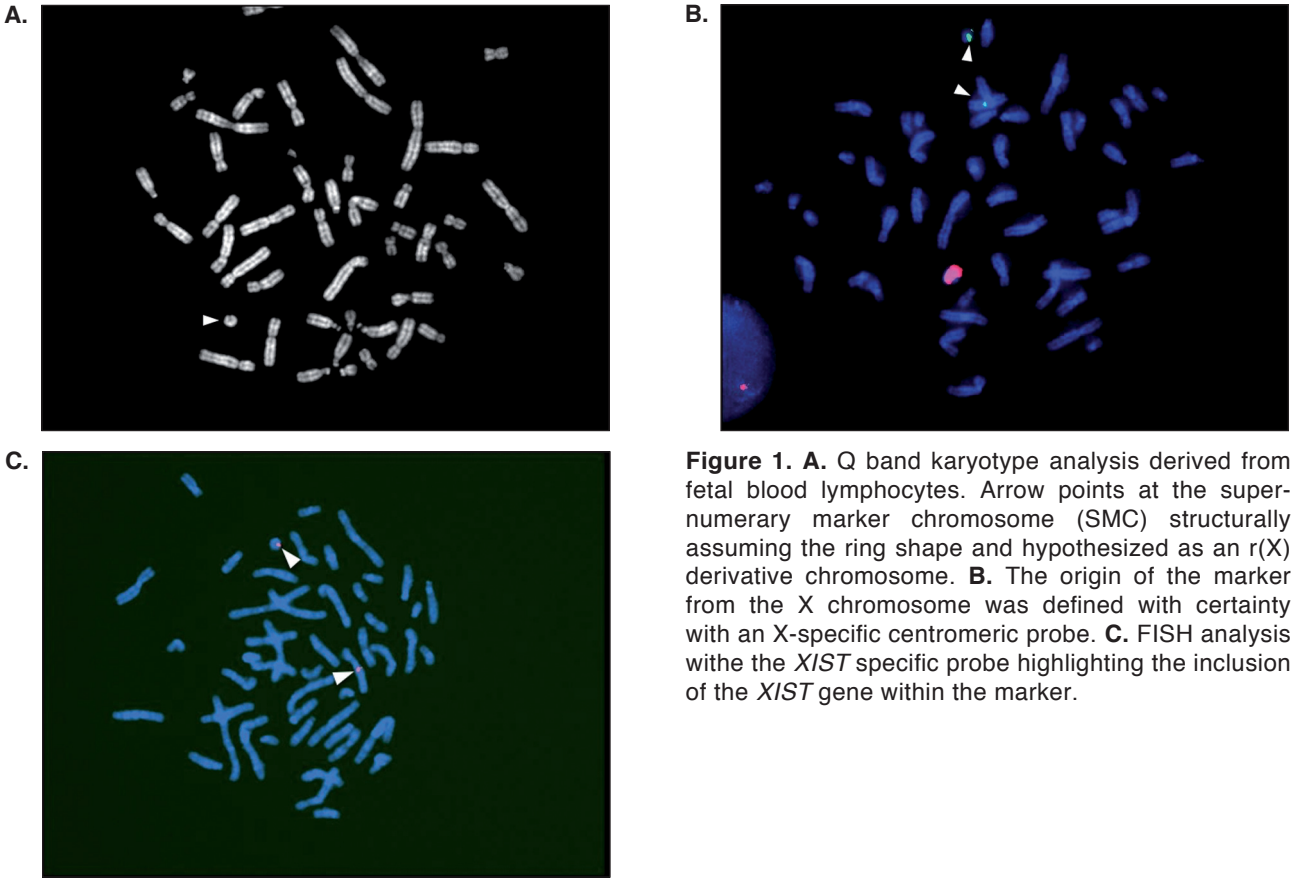

Figure 1. A. Q band karyotype analysis derived from fetal blood lymphocytes. Arrow points at the supernumerary marker chromosome (SMC) structurally assuming the ring shape and hypothesized as an r(X) derivative chromosome. **B.** The origin of the marker from the X chromosome was defined with certainty with an X-specific centromeric probe. **C.** FISH analysis withe the *XIST* specific probe highlighting the inclusion of the *XIST* gene within the marker.

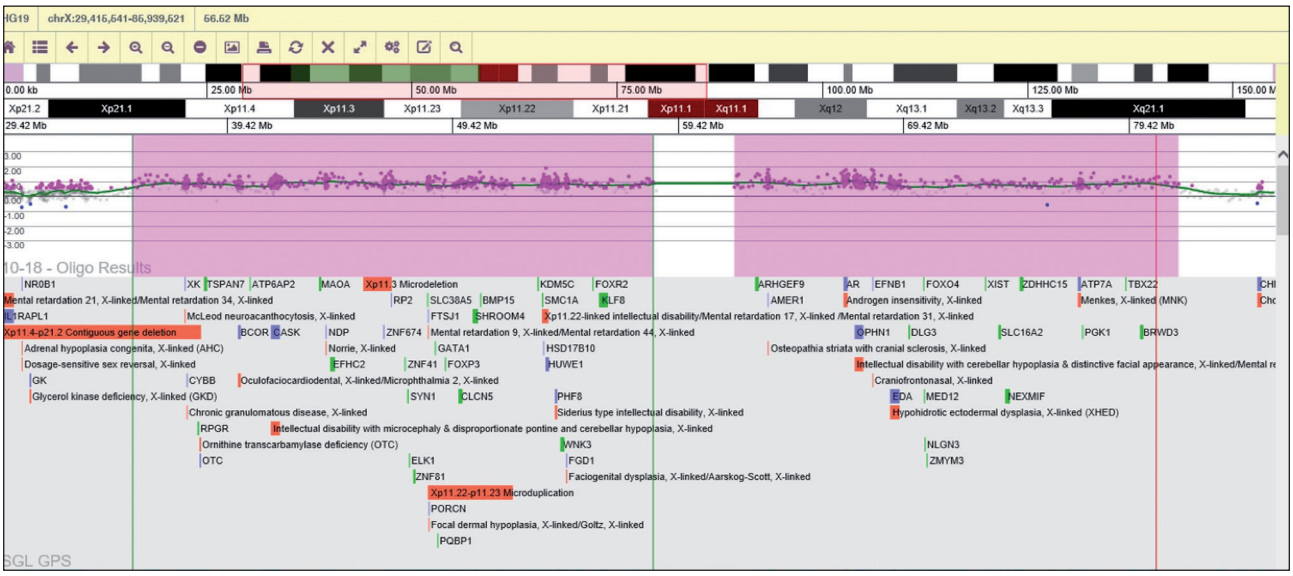

Figure 2. CGH array output showing the extension of the duplication (pink color) along the chromosome X and the OMIM genes contained within, including the *XIST* gene.

Discussion

In general, the consequences of the presence of an undefined SMC are extremely variable.

In about half of the cases, it is symptomless; in others, it can be associated with relevant clinical symptoms, such as growth retardation, intellectual disability or complex malformation patterns [17-20].

Non-satellited *de novo* undefined markers present in all cells carry an empiric risk of about 14% of possible structural anomalies and/or intellectual disability in the postnatal life [21].

In our case, the risk estimate could be only slightly reduced by the mosaic distribution of the SMC, as the distribution and the consequent functional outcomes are not predictable.

However, in our case, by karyotyping fetal blood and by FISH and CGH array analysis, we were able to establish that the SMC was indeed a ring derived from the X-chromosome and carried a 43Mb duplication of the X chromosome.

While entire extra X-chromosomes are generally well tolerated, as exemplified by the 47,XXX or 47,XXY syndromes, smaller derivative X-chromosomes can be paradoxically more development detrimental [22-25].

The pathological outcome is a consequence of the loss of the *XIST* gene and the ensuing unbalanced expression of a set of X-linked genes [26-28]. Hence, in the context of an r(X), the most important issue, conditioning the prognosis, is the inclusion or exclusion of the *XIST* gene within the duplicated X-fragment.

In our case, CGH and FISH analysis with an *XIST* specific probe confirmed the inclusion of the *XIST* gene in the sequences of the duplicated fragment, predicting a normal occurrence of the inactivation of the supplementary set of genes included in the r(X). However, searching the literature for similar cases, we did not find any report of r(X) with preserved *XIST*. Only a few cases of males with X-chromosome rings are described and all were missing the *XIST* gene, assessed by FISH analysis. Furthermore, they were all smaller than 43 Mb and none of them, but one, was fully characterized by CGH array [9].

Hence, in the latter cases, the abnormal outcome is likely dependent on the absence of the *XIST* gene and impairing of the inactivation of the r(X). In fact, in all reports of the literature but one, r(X)s lacking the *XIST* gene in males result in the absence of X inactivation and pathological neonatal outcomes [10-13].

The prenatal finding of an r(X) chromosome lacking the *XIST* gene implies a high risk of phenotypic anomalies in both sexes. Males with r(X) and preserved *XIST* gene are rarely found, likely because, developing normally, they escape clinical detection. Accordingly, in the literature there are almost exclusive descriptions of r(X) mosaic males with abnormal phenotype associated with the absence of the *XIST* gene. The pathological phenotype is typically characterized by facial dysmorphisms (hypertelorism, sunken nasal root, anteverted nostrils) and other anomalies (microcephaly, urogenital anomalies, limb and CNS abnormalities) [10, 12, 13].

In the literature, there is only one report of *XIST* negative r(X) in a male with a normal outcome at one-year follow-up [11]. The normal phenotype in this male was likely dependent on the small size of the duplicated X fragment and/or on a favorable tissue distribution of the mosaic r(X) line.

In the case of the fetus reported here, the presence of *XIST* gene suggests that the ring is theoretically subject to random inactivation, which could be comforting, but considering the extreme instability of the ring, we cannot be sure that the ring maintains the *XIST* gene in all cell divisions or can express it in all tissues. Since the gene dosage is regulated by the random inactivation of this r(X), the lack of inactivation could lead to an unbalanced dosage of those X-linked genes present in the extra r(X). Even though the morphological ultrasound analysis, performed later in pregnancy, was reassuring in showing a male fetus normal for biometrics and anatomical structure, in the absence of reports of comparable cases with known favorable outcome, we could not completely reassure the prospective parents on the normal postnatal outcome of the fetus and exclude potential intellectual disability.

Despite this uncertainty, based mainly on the theoretical assumption of the prevalent inactivation of the r(X) and on the absence of ultrasound anomalies, the couple continued pregnancy, giving birth to a normal neonate. The baby's follow-up until 13 months of age does not show any anatomic or functional anomaly with normal psychomotor development as assessed by the Denver scale of child development. If confirmed by other reports, our first description of a normal postnatal outcome in a male fetus with mosaic r(X) and retained *XIST* should encourage toward a conservative choice other parents of fetuses with similar prenatally discovered r(X) and absence of ultrasound anomalies during pregnancy.

Declaration of interest

The Authors declare no conflict of interest.

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