A case report of a meiotic segregation study on a small supernumerary marker chromosome

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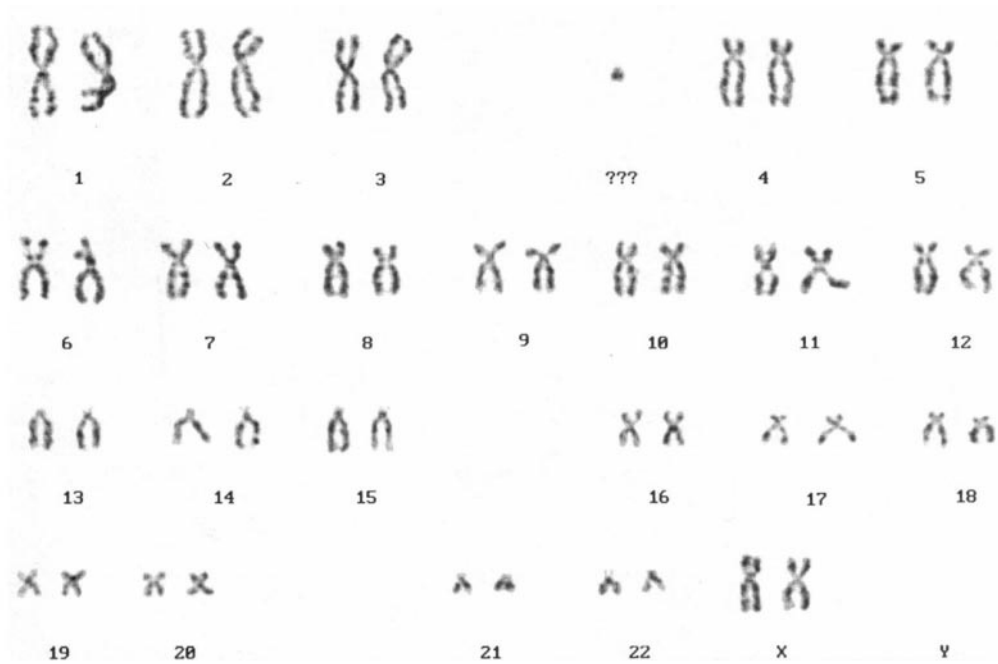
Summary

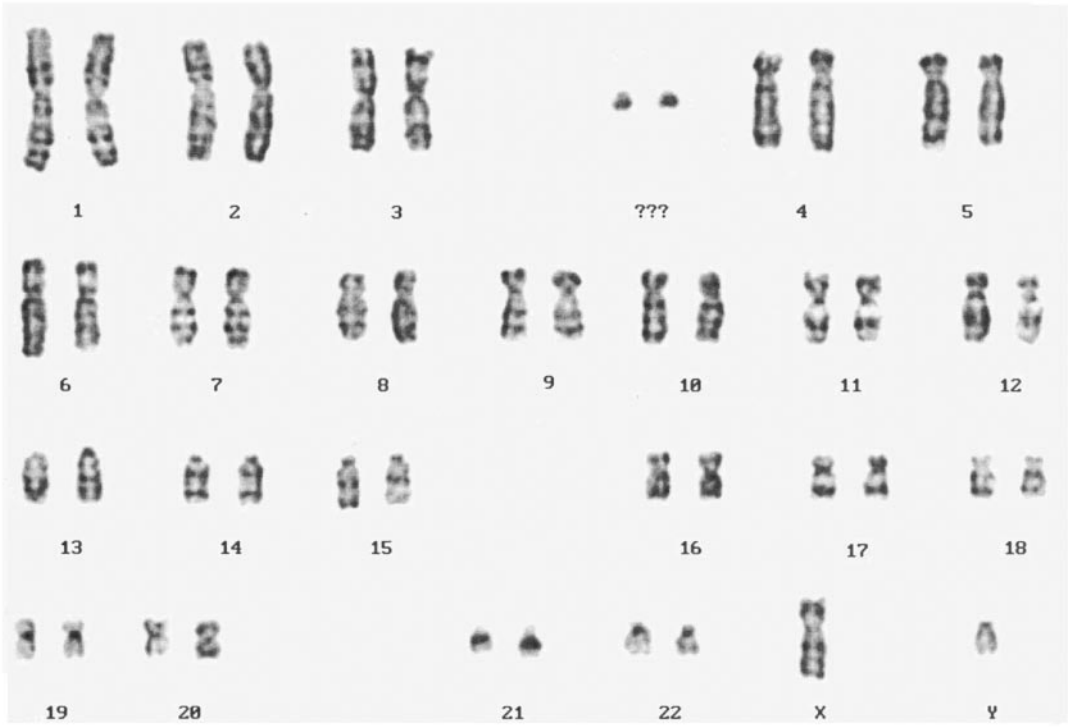
Small supernumerary marker chromosomes (sSMCs) have been described from all human chromosomes with different sizes and shapes. However, it is difficult to know the clinical manifestations associated with them, because such knowledge depends on the size, presence of euchromatic material, degree of mosaicism and/or uniparental disomy (UPD).
A case report of a familial small supernumerary marker chromosome (sSMC) through a structural and a segregation study is reported.

Case report

A particular class of observable trisomies in the study of the human karyotype is constituted from the presence of small supernumerary marker chromosomes (sSMCs) (1). sSMC are structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone, and are (in general) equal in size or smaller than a chromosome 20 of the same metaphase spread.
Generally the marker chromosomes respond to three different structural typologies: those that have a unique centromere (monocentric), those that have two centromeres (dicentric) and those without centromere (acentric) (2) e analyzed the case of a man whose karyotype results to be 48,XY,+2mar (Fig. 1), condition found following the analysis of follow-up on a foetus with karyotype 47,XX,+mar (Fig. 2) to such purpose we tried to characterize at structural level and to define the mechanism of segregation in at least three generations by Fluorescent In Situ Hybridization (FISH) (3). When a chromosomal abnormality after foetal karyotyping is observed, the analysis of the karyotype of the family components is mandatory to understand if the presence of the sSMCs is to be consider "*de novo*" mutation or transmitted through the germinal line.
Until today a lot of methods were used to characterize the nature of sSMCs in different kind of tissues. We analysed with classical cytogenetics method karyotypes of the individuals of three generations (fatherly grandmother, father, mother and foetus) with the purpose to define a scheme of distribution of the sSMCs.
A FISH analysis was made on the available sample: amniotic fluid (foetus), seminal liquid (father), peripheral blood (father, mother and fatherly grandmother) prepared as described by standard protocols (4). The hybridization has been effected with Hybrite as described by factory protocols (Vysis).
In our case it was not been possible to characterize the sSMC by the usual GTG banding because of its small dimensions.
The results of the study of the karyotypes obtained by peripheral blood of the parents of the foetus and on the fatherly grandmother brought the followings results (Fig. 1):
– maternal karyotype: 46,XX
– fatherly karyotype: 48,XY,+2mar
– karyotype fatherly grandmother: 47,XX,+mar.
The FISH analysis has been used for characterizing the molecular nature of the marker.
In our case positiveness of hybridization was for the probe LSI D15S11 SpectrumOrange-CEP15 SpectrumGreen Vysis in all samples. In such hybridization both centromeric probes and euchromatic subcentromeric probes were seen. In all the analyzed samples, excluded that fatherly, a trisomy of the centromeric region has been found (Fig. 2; FISH on foetal karyotype), while in the FISH analysis on fatherly blood a tetrasomy of such region (Fig. 2; FISH on father's karyotype) has been found. A double positivity for hybridization was found for the subcentromeric region, for all the analyzed samples. To understand as the chromosome marker, derivative of the chromosome 15, could have segregated during the spermatogenesis, two different analyses have been performed using FISH on nucleuses interfasic on fatherly sperms. The first one using probes centromeric CEP15 SpectrumGreen Vysis and CEP12 SpectrumOrange Vysis (Fig. 3; first image). From the picked data he can clearly deduce a condition of apparent mosaicism for the chromosome 15 with the 61% of monosomy, the 37% of disomy and him 1% of trisomy (Tab. I). The second hy-

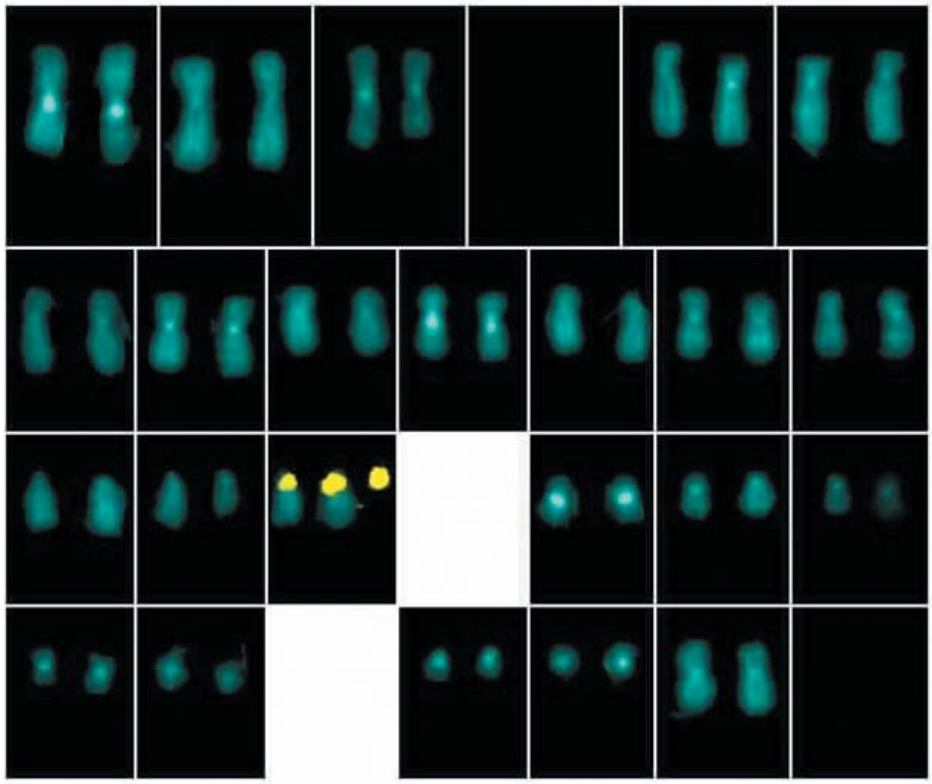


Foetal Karyotype

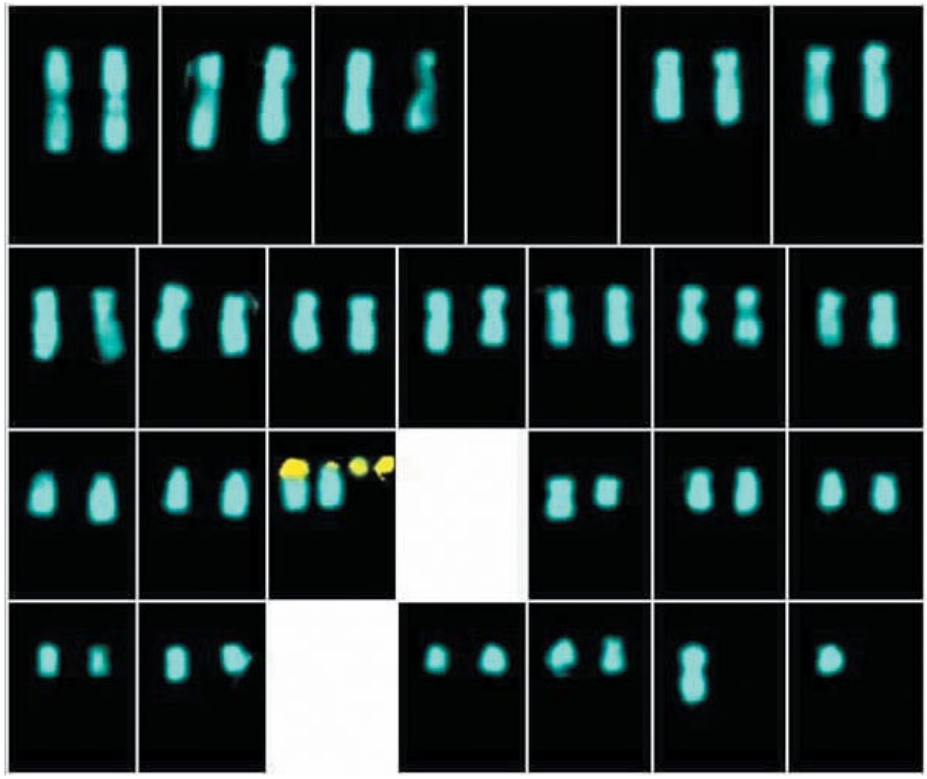


Father's Karyotype

Figure 1



Foetal Karyotype with FISH for CEP15 and DAPI counterstaining



Father's Karyotype with FISH for CEP15 and DAPI counterstaining

Figure 2

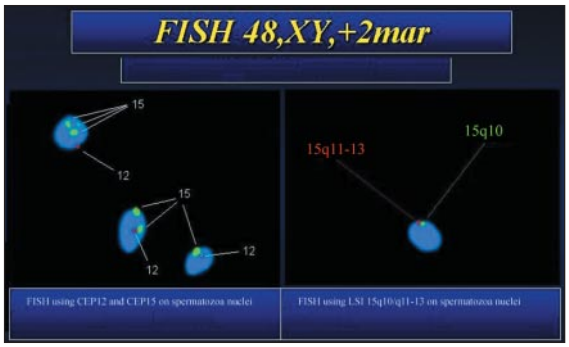


Figure 3

Table I

	Crom. 12	Crom. 15
Monosomy	3920/4000 98%	2456/4000 61%
Nullisomy	0	5/4000 1%
Disomy	800/4000 2%	1493/4000 37%
Trisomy	0	50/4000 1%

bridization has been effected using the probe LSI D15S11 used for the other tissue analyzed in precedence (Fig. 3; second image).The results mostly show an exhaustive vision of as is distributed the positivity for hybridization on the analyzed nucleuses.In fact inside the monosomic population is observed a 44% of containing nucleuses the whole chromosome 15, while the remainder 17% is constituted by nucleuses with the only marker. Therefore it can be inferred that the relationship between the segregation of the chromosome marker against the chromosome 15 is of around 1:4 (Tab. II). In the disomic population (initially esteemed to 37%) was observed a 23,5% of nucleuses containing a whole chromosome 15 and an SSMC (15q10+/15q11-13+/15q10+/15q11-13-); while 10,4% were nucleuses with two SSMCs (15q10+/15q11-13-); the remainder 3,2% consists instead of two whole chromosomes 15 (15q10+/15q11-13+). Finally in the trisomic population, however found in low frequency, a mixed population of nucleuses was found with two whole chromosomes 15 and an SSMC and vice versa (0,5% each).

Considering all these data we deduced that the total degree of segregation will results from the sum of the different percentage of segregation related to the different populations: 17% + 23,5% + 10,4% + 0,5% + 0,5% = 52%.

Among the nucleuses where the SSMC is present a degree monosomy vs. disomy of 1:4 (10,4% + 0,5% and 17% + 23,5% + 0,5% respectively) has been found.

Discussion

Considering the data from classical cytogenetics studies made on peripheral blood of different individuals, it would be attend for a high degree of disomy in the spermatic cells of the individual 48,XY,+2mar. Therefore the further observations of molecular cytogenetics have allowed us to observe a prevalence of monosomic spermatic cells (61%) and a lower degree of disomic cells (37%). This mosaicism is presumably explainable with a strong instability of the pairing complex of the four SSMCs during the meiotic prophase. Besides such observations there are no data for establishing if there are eventually potential evolutionary pressures during the various stages of the male gametogenesis.

Table II

15q10	15q11-13			
	0 spots	1 spots	2 spots	3 spots
1 spot	260/1530 (17%)	680/1530 (44.5%)	0	0
2 spots	160/1530 (10.5%)	360/1530 (23.5%)	50/1530 (3.2%)	0
3 spots	0	7/1530 (0.5%)	7/1530 (0.5%)	0

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