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Complex sSMC Involving X and Y Chromosomes in two Patients with 45,X/46,X,+mar Karyotype

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Abstract- **Complex small supernumerary marker chromosomes (sSMCs) consist of chromosomal material derived from two or more different chromosomal regions and constitute one of the smallest subsets of sSMC. Most of complex sSMCs are represented by a der(22)t(11;22) in Emanuel syndrome. As far as we know, only one recent report has described sSMCs involving simultaneously X and Y chromosomes in Turner Syndrome.**

We report two patients, a female and a male, both with a complex sSMC derived from X and Y chromosomes in mosaic with a 45,X cell line. In both patients, the marker chromosomes were early replicating and the *XIST* gene was absent. FISH and PCR confirmed the presence of Yp loci (*TSPY, AMGY, SRY,* DYZ3), and negative for DYZ1. The DAZ4 sequence was present only in patient 1. Our findings suggested that complex sSMC involving X and Y chromosome could be a kind of sSMC of the gonosomes.

Keywords: molecular cytogenetics, mosaicism. sex chromosomes, complex small supernumerary marker chromosome, turner syndrome.

I. Introduction/background

Complex small supernumerary marker chromosome (sSMC) consist of chromosomal material derived from two or more different chromosomal regions (Liehr, 2012). sSMC are only identifiable by molecular cytogenetic analysis, because their size and the variability of involved chromosomal regions (Trifonov *et al*., 2008). The characterization of the structure, regions and genes involved in the sSMC are important for the genotype-phenotype correlation (Liehr, 2012).

Complex sSMC constitute 8.4% of all sSMC, and are observed mainly in Emanuel syndrome (ES; 82.2%) (Liehr *et al*., 2013). Fewer than 100 cases are known (Liehr *et al*., 2013). Excluding ES cases, as they are difficult to identify and their frequency is underestimated (Trifonov *et al*., 2008; Liehr *et al*., 2013).

Parental studies in 57 complex sSMCs (excluding ES) showed that 36% of them were *de novo*, and the remainder (64%) were inherited from a balanced translocation in one parent. Mosaic cases with karyotype 47,XN,+mar/46,XN were only seen in *de novo* complex sSMCs (Liehr *et al*., 2013).

sSMC can be present in numerically abnormal karyotype like in a Turner syndrome (TS) karyotype (45,X/46,X,+mar), leading to female or male phenotypes (Liehr *et al*., 2007; Wang *et al*., 2017). In TS, the sSMC are derived from one of the gonosomes in more than 99% of the cases; there are also exceptional reports on sSMC derived from autosomes (Liehr *et al*., 2007; Wang *et al*., 2017; Sheth *et al*., 2009; Jafari-Ghahfarokhi *et al*., 2015).

Recently, a complex sSMC from X and Y chromosomes have been described in a Turner syndrome (Li *et al*, 2020). Here we report two mosaic patients, a TS patient and an unidentified syndrome male, with a 45,X cell line and a cell line with complex sSMC involving X and Y chromosomes, characterized by Fluorescence *in situ* hybridization (FISH) and Polymerase Chain Reaction (PCR).

II. Methods

Patient 1 (P1) come from a cohort of 21 TS patients with marker chromosomes, and Patient 2 (P2) from another cohort of 19 patients with uncharacterized marker chromosomes, evaluated in Cytogenetic Laboratory of IPPMG, UFRJ. The informed consent was obtained from the patients or their parents (Approved by the Ethics Committee of IPPMG/UFRJ nº 13/09).

Chromosomes were examined using G banding and differential replication staining (late BrdU labelling). Fluorescence *in situ* hybridization (FISH) were performed using commercial probes: Whole Chromosome Painting (wcp) X and Y, XYpter and XYqter, *SHOX* (Xp22 and Yp11.3), *KAL1* and *STS* (Xp22.3); *XIST* (Xq13.2), DYZ3 (Yp11.1-q11.1), *SRY*

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(Yp11.31) and DYZ1 (Yq12), according manufacturers' instructions.

Genomic DNA was isolated from peripheral blood using a commercial DNA isolation kit and the polymerase chain reaction (PCR) was performed using six primers sets for Y-chromosome-specific sequences: *SRY* (Yp11.31), *TSPY1* (Yp11.2), *AMGY* (Yp11.2), *DAZ4* (Yq11.23), DYZ3 (Yp10-q10) and DYZ1 (Yq12).

III. Clinical Informations

P1: female, referred at 7 years of age due to short stature. First child of an unrelated couple, a young mother and an unknown father. Vaginal delivered at 40th week gestation; birth weight of 2.6kg and birth length of 48cm. She developed short stature, developmental delay and intellectual disability. Menarche was induced at 17th year. On physical examination at 30th year she presented: short stature (145cm; not treated with growth hormone), relative macrocephaly, ocular hypertelorism, high-arched palate, short neck, low posterior hairline, shield shaped thorax, widely spaced nipples, cubitus valgus, multiple pigmented nevi, hyperconvex nails, hypoplasia of the second toe, bicuspid aortic valve and obesity (Fig. 1a) and a typically female external genitalia. Ultrasound examination showed reduced uterus and unidentified ovaries. Prophylactic gonadectomy was recommended.

P2: male, referred at 4 years of age due to neuropsicomotor developmental delay, autistic behaviour, aggressiveness and hyperactivity. First child of a healthy and unrelated young couple. Maternal thrombocytopenia. Vaginal delivered at 38th week gestation; birth weight of 2.3Kg, birth length of 47cm and head circumference of 32cm. He didn't walk until his 15th month of age. Speech delay was evident by 2 years of age. Recurrent episodes of pneumonia. On physical examination at 8 years, he presented triangular face, ocular hypertelorism, arched eyebrows, long eyelashes, long palpebral fissures, high-arched palate, diastema, widely spaced nipples, single transverse palmar crease (Fig. 1b) and a typically male external genitalia (normal scrotum, palpable testes and a normal sized penis). Ultrasound examination showed normal prostate size and absente Müllerian remnants. No specific syndrome could be related to this patient clinical symptoms.



Figure 1: Appearance of P1 and P2: a) P1: 30-years-old, woman showing minor facial dysmorphic features and hypoplasia of the second toe. b) P2: 8-years-old, boy phenotype showing minor facial dysmorphic features, ocular hypertelorism and bilateral transverse palmar crease.

P1: Karyotype was 45,X/46,X,+mar; the marker chromosome was a dicentric sSMC, with early replication, and alternating morphology. The mother presented normal karyotype.

The sSMC was positive for both X and Y with wcp, and presented two copies of XYpter, DYZ3, *SRY* and *SHOX*, one copy of *KAL1* and *STS*; was negative for *XIST*, DYZ1 and XYqter (Fig. 2). The wcp analysis also showed the presence of cryptic cell populations, one with the presence of an sSMC derived from chromosome X(wcpX+) and another with a sSMC derived from chromosome Y(wcpY+), but the frequency of these cells was too low to be determined. The frequency of nuclei with two DXZ1 signals was 1,7%. In each metaphase only one sSMC was observed.

PCR was positive for *TSPY1, AMGY, SRY,* DYZ3 and *DAZ4,* and negative for DYZ1.

The redefined karyotype was: mos 45,X/46,X,+mar.ish.der(X;Y)(DYZ3++,SHOX++,SRY++,KAL1+,XYpter++,wcpX+,wcpY+,XIST-,STS-, DXZ1-,DYZ1-)

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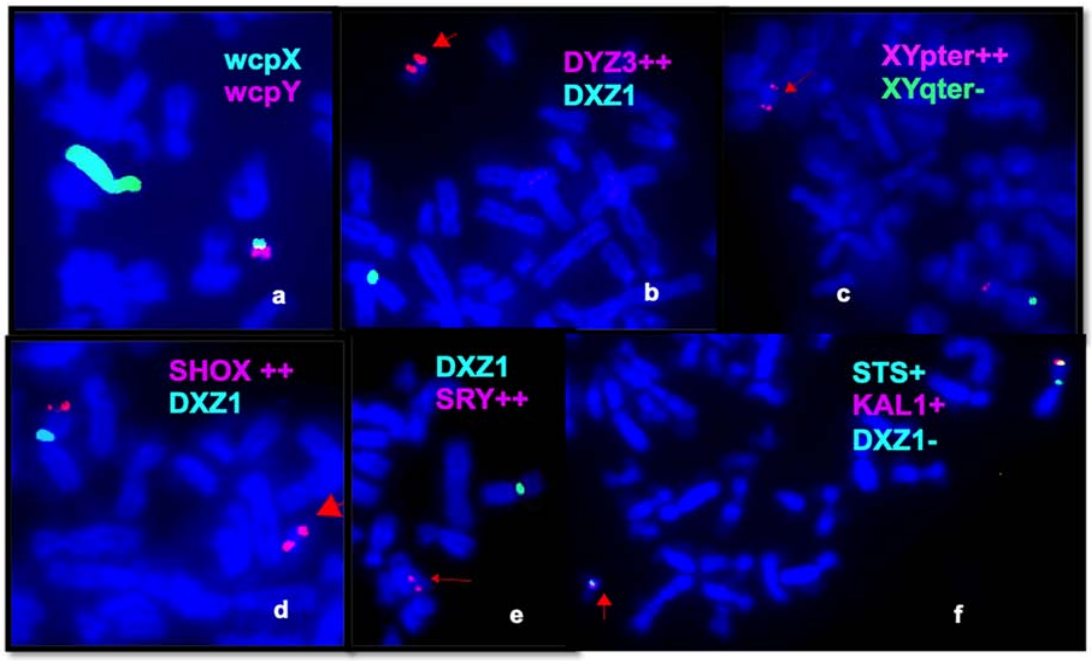


Figure 2: FISH results for P1. Partial metaphases showing the presence of: a) both signals of wcpX and wcpY (red arrow) indicating the participation of X and Y chromosomes in complex sSMC; b) two copies of DYZ3 (red arrow); c) two copies of XYpter (red arrow); d) two copies of SHOX (red arrow); e) two copies of SRY (red arrow) and f) one copy of STS/ KAL1 (red arrow).

P2: Karyotype was 45,X/46,X,+mar, the marker chromosome was a *de novo* ring sSMC, early replicating. Both parents presented normal karyotype.

The sSMC was positive simultaneously for X and Y with wcp, and presented one copy of XYpter, DYZ3, *SRY*, *SHOX*, and *KAL1*; it was negative for *XIST*, *STS*, DYZ1(Yq12) and XYqter (Fig. 3). Sometimes the sSMC appeared to be dicentric.

PCR was positive for *TSPY, AMGY*, *SRY*, DYZ3 and negative for DAZ4 and DYZ1.

The redefined karyotype was: mos 45,X/46,X,+mar.ish.der(X;Y)(DYZ3+,SHOX+,SRY+,KAL1+,XYpter+,wcpX+,wcpY+,XIST-,STS-,DXZ1-,DYZ1-)

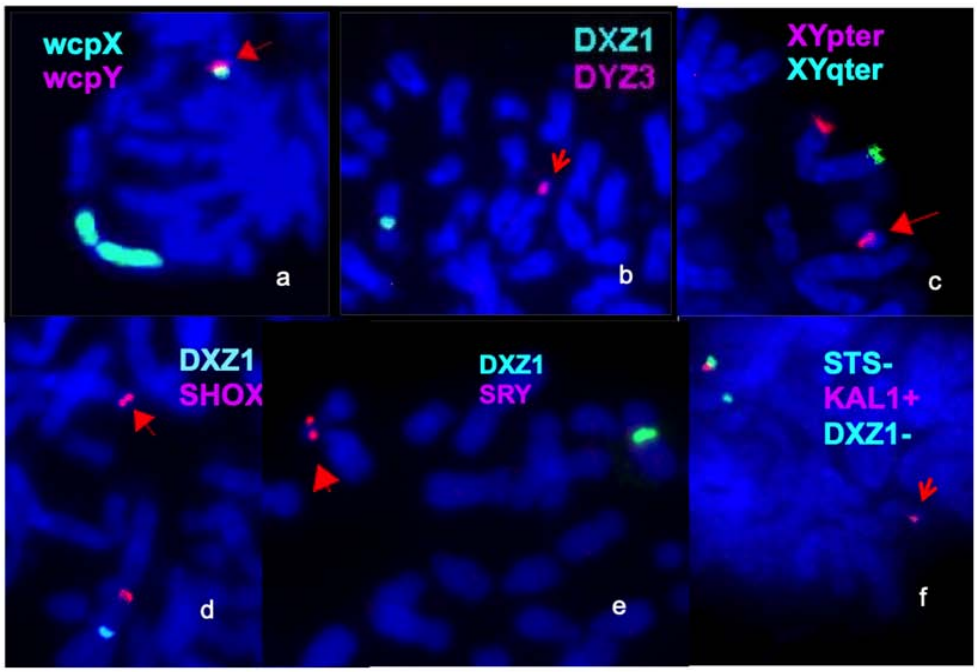


Figure 3: FISH results for P2. Partial metaphases showing the presence of: a) both signals of wcpX and wcpY (red arrow) indicating the participation of X and Y chromosomes in complex sSMC; b) DYZ3 (red arrow); c) XYpter (red arrow); d) SHOX (red arrow); e) SRY (red arrow) and f) KAL1(red arrow).

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IV. Discussion and Conclusion

We report two original cases of complex sSMC, a TS patient and a unidentified syndrome male patient involving X and Y chromosomes, both mosaic with a 45,X cell line. Molecular techniques were crucial to determine the presence of the Y chromosome material in these patients. The presence of Y chromosome segments could increase the risk for gonadoblastoma. Prophylactic gonadectomy is recommended by expert consensus in TS patients with euchromatic Y-chromosome, due to an increased risk (around 10%) of gonadoblastoma (Gravholt *et al*., 2017). The gonadectomy was recommended to P1.

In P2, the sex differentiation and a normal male external genital were possible because of the presence of *SRY* gene, despite of a 45,X lineage. The clinical variability could be strongly influenced by the concentration and distribution of the 45,X cell line in the various tissues, and the differential expression of genes located on the Y chromosome (Patsalis *et al*, 2005; Lindhardt *et al*., 2012). Males with a 45,X/46,XY karyotype and its variants seem to have a strong chance of normal testicular function (Lindhardt *et al*., 2012). However, the association of the phenotypic characteristics with the presence or absence of Y-chromosomal loci, hosting genes other than *SRY* remains uncertain (Patsalis *et al*, 2005; Lindhardt *et al*., 2012). In both patients, the absence of *XIST* on sSMC, and the early replication suggested that the sSMC was not inactivated. This may lead to different clinical outcomes, especially about mental development (Liehr *et al*., 2007). Studies in TS females have indicated that a severe phenotype and intellectual disability could be primarily caused by active partial X disomy resulting from the deletion or impaired expression of the *XIST* (Migeon *et al*., 2000).

Complex sSMC of P1 had two copies of *SHOX* gene, and patient 2 one copy. *SHOX* haploinsufficiency have been associated with short stature and various skeletal features in TS patients, such as scoliosis, high arched-palate, and micrognathia (Li *et al*., 2017). The short stature in P1 should be due the presence of a 45,X cell line.

Complex cryptic mosaicism for sSMC derived from chromosome X has been described earlier (Liehr, 2012; Santos *et al*., 2010]. Some Y-chromosome microdeletions in critical regions could provide instability on the Y-chromosome leading to the development of a 45,X cell line (Patsalis *et al*., 2005). Adikusuma *et al.* (2017) using CRISPR/Cas9 technology to remove Y chromosome sequences showed that both centromere removal and chromosome shredding induced Y chromosome loss. In both P1 and P2, the rearrangement occurred near the pseudoautosomal region; this region could be prone to rearrangements because of its sequence homology. Structural chromosome rearrangements involving both X and Y chromosomes are very unusual (Bispo *et al*., 2014). Liehr *et al.* (2013) reviewed 73 complex sSMC (excluding ES), which only three were derived from sex chromosomes, one with material from X chromosome and two with material from the Y chromosome. Although complex markers represent a small percentage (~0,9%) of sSMCs, this may be underestimated as highlighted in recent studies applying aCGH (Reddy *et al*., 2013). A complex sSMC involving both X and Y chromosomes in a TS patient in a group of 75 marker chromosomes, was reported (Li *et al*, 2020).

We present the cytomolecular characterization of two original mosaicism cases with 45,X cell lines and a complex sSMCs involving X and Y chromosomes. These findings suggest that complex sSMCs involving X and Y chromosomes could be much more frequent than previously described (Bispo *et al*., 2014).

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Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

Author Contributions

MBG performed the FISH and replication experiments of patient 1, interpreted the results drafted and the initial manuscript. MOF performed the FISH and replication experiments of patient 2 and interpreted the results. ISP performed PCR. ISP, EK, MMG and MGR did patient' clinical diagnosis and treatment. SAPP performed G-banding analysis. MCMR reviewed all laboratory results, participated in its design and coordination, and helped draft the initial manuscript. MGR participated in its design and coordination, and helped draft the initial manuscript.

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