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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.

Index terms— molecular cytogenetics, mosaicism. sex chromosomes, complex small supernumerary marker chromosome, turner syndrome.

1 I.

Introduction/background omplex 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).

2 II.

3 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 C 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). 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).

4 Medical Research

5 III.

6 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 40 th 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 17 th 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. 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-)

Complex sSMC Involving X and Y Chromosomes in two Patients with 45,X/46,X,+mar Karyotype 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 38 th 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. 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-)

7 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 Ychromosome, 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 Ychromosomal 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). 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. [1] [2]

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

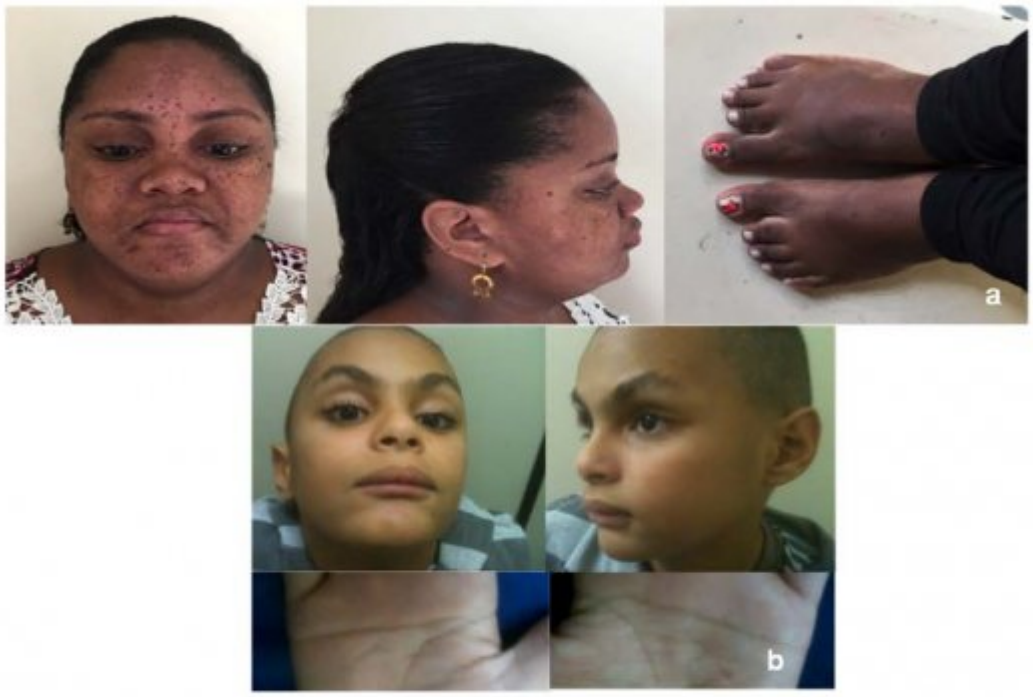

Figure 1:

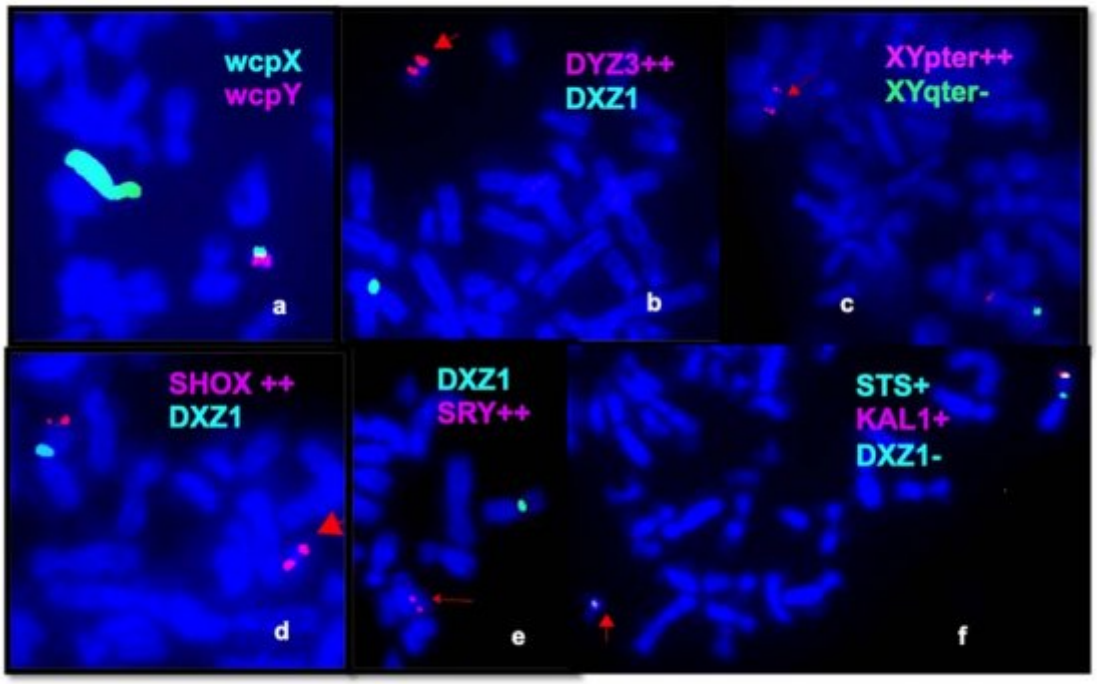

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Figure 2: Figure 1 :

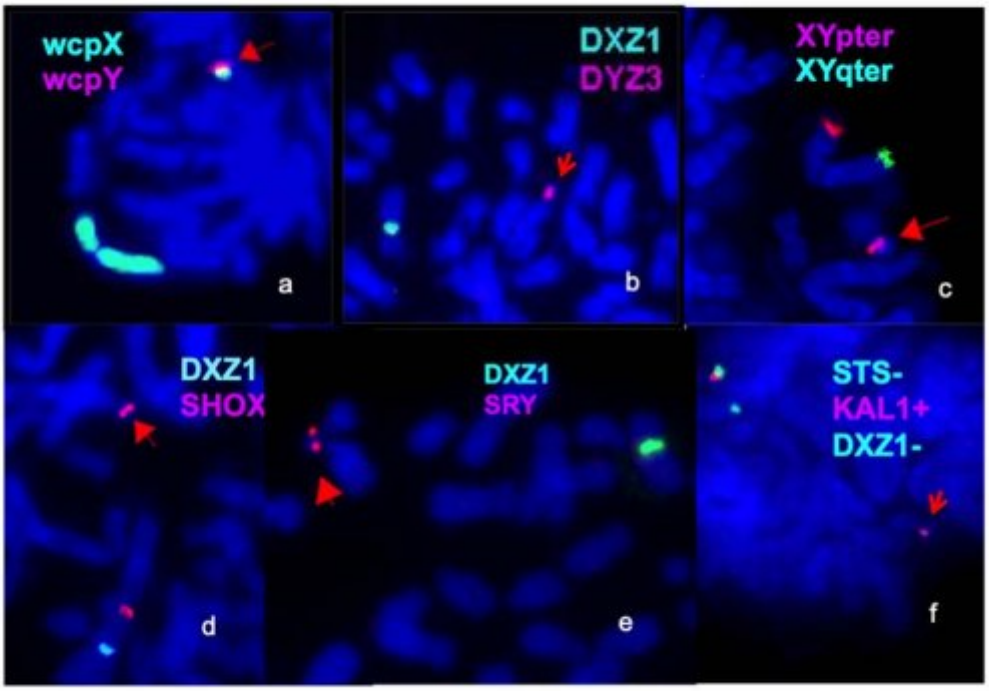

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Figure 3: Figure 2 :

.1 Acknowledgments

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.2 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

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