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Research Letter

Prenatal diagnosis and molecular cytogenetic characterization of a small marker chromosome derived from Y chromosome

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A 37-year-old, gravida 2, para 1 woman underwent amniocentesis at 17 gestational weeks because of advanced maternal age. Cytogenetic analysis of amniocytes revealed a small marker (mar) chromosome (sMC) and monosomy X in eight colonies and a karyotype of 46,XY in 16 colonies (Fig. 1). The parental karyotypes were normal. Prenatal ultrasound revealed a normal singleton fetus with normally developed male external genitalia. The sMC was characterized by spectral karyotyping (SKY; Applied Spectral Imaging, Carlsbad, CA, USA), fluorescence *in situ* hybridization (FISH), multicolor banding (MCB; MetaSystem, Altlussheim, Germany), and array-based comparative genomic hybridization (aCGH). SKY analysis revealed that the sMC originated from the Y chromosome (Fig. 2). FISH analysis using DNA probes, such as an X-centromere α-satellite probe of chromosome enumeration probe X (*DXZ1*) (Xp11.1-q11.1); TelVysion Xp/Yp (Xpter/Ypter) and Xq/Yq (Xqter/Yqter) telomeric probes; an *SRY* probe of LSI SRY (Yp11.31); a Y-centromere α-satellite DNA probe of CEPY (*DYZ3*) (Yp11.1-q11.1); and a Y long-arm α-satellite DNA probe of CEPY (*DYZ1*) (Yq12) (Abbott, Abbott Park, IL, USA), showed that the sMC contained *SRY* and *DYZ3* signals but lacked Yp and Yq telomeric signals and *DYZ1* signal (Fig. 3). MCB analysis showed that the sMC was a ring Y chromosome, or r(Y), consisting of Yp and centromere (Fig. 4). The result of aCGH was consistent with those of FISH and MCB. The karyotype was 46,X,r(Y)(::p11.31→q11.1::)(Ypter-, *SRY*+, *DYZ3*+, *DYZ1*-, Yqter-, mBand Y+, SKY+)[8]/46,XY [16] (Figs. 5 and 6). The parents decided to continue the pregnancy, and a 3,200-g normal male baby was delivered with normal male external genitalia.

Prenatal diagnosis of sMCs gives rise to difficulties in genetic counseling and requires molecular cytogenetic technologies to identify the nature of the aberrant chromosome [1–5]. The present study reports a case of mosaicism for a small derivative Y chromosome presenting as an alphoid ring Y chromosome derived from Yp.

Prenatal diagnosis of a small derivative Y chromosome should prompt a FISH study to make a differential diagnosis of an analphoid sMC. Analphoid, acentric, or neocentric sMCs lack centromeric heterochromatin and α-satellite DNA and have been proposed to contain neocentromeres. Neocentromeres are formed within interstitial chromosomal sites that have not previously been known to express centromere function [6]. Analphoid supernumerary sMCs are usually small inverted duplication (inv dup) chromosomes [7,8].

sMCs with neocentromere formation in Yp have been previously described [9,10]. Conde et al [9] reported a newborn baby with intrauterine growth restriction;

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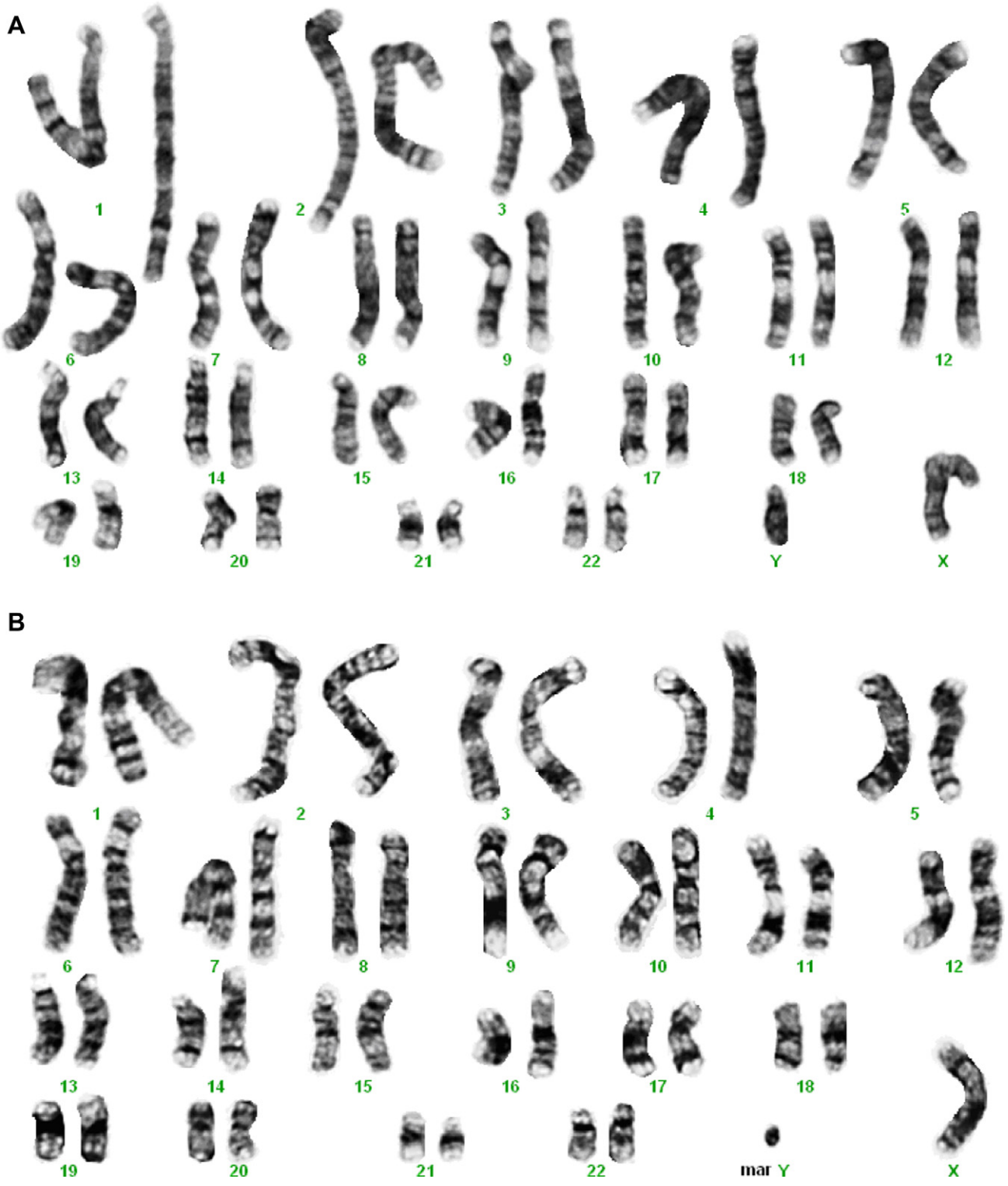

Fig. 1. (A) A karyotype of 46,XY and (B) a karyotype of 46,X,r(Y).

ambiguous genitalia; a phallus resembling a clitoris; a perineal urethral orifice; pseudovaginal labioscrotal hypospadias; hyperpigmented labioscrotal fold with gonads; mild hydrometrocolpos; presence of uterus and vagina but no gonads on pelvic ultrasound; a 45,X cell line in 85% of lymphocytes; and an *SRY*-positive analphoid mar(Y) cell line in 15% of lymphocytes. On further evaluation, the tissue samples showed an 85% cell line of 45,X; a 9% cell line of an acentric mar(Yp) with one copy of *SRY*; and a 6% cell line of an acentric mar(Yp) with two copies of *SRY*. Sheth et al [10]

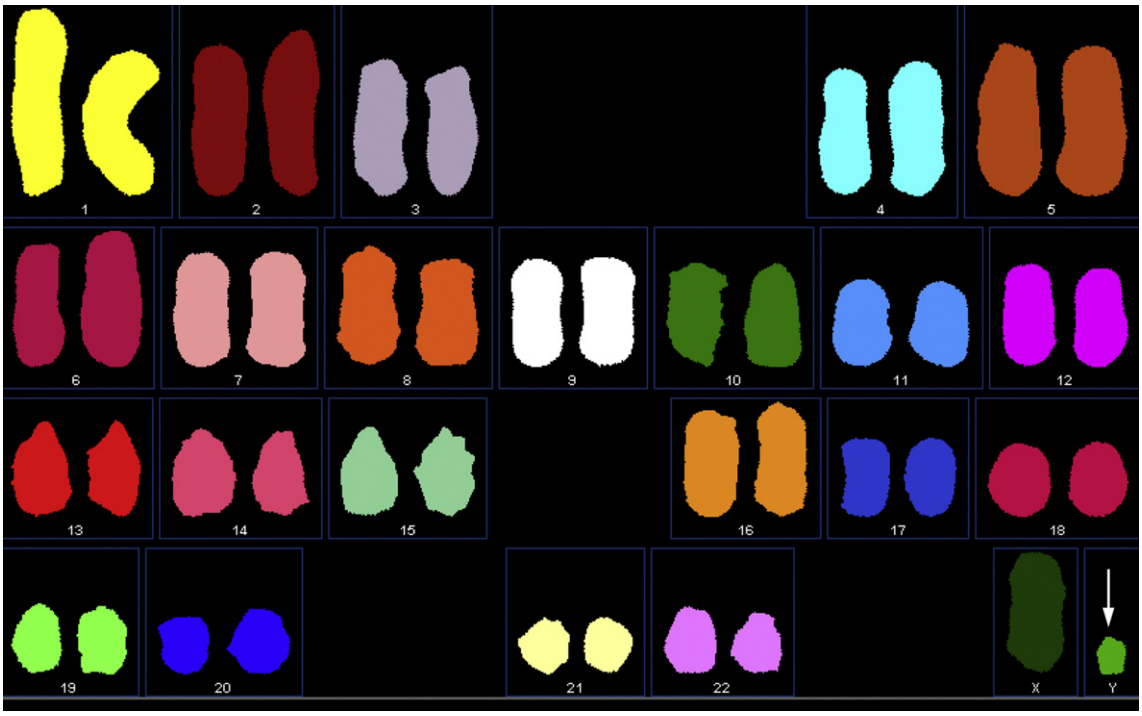

Fig. 2. Spectral karyotyping analysis shows a Y-derived small marker chromosome (arrow).

reported a neocentric isochromosome Yp derived from an inv dup(Yp) presenting in a karyotype of 47,XX,+inv dup(Y)(pter→Yp11.2::Yp11.2→pter) detected at amniocentesis. The fetus manifested as a phenotypically normal male. In a study of 12 cases of inv dup mars, Murmann et al [11] found that all were formed from an acentric fragment after a double-strand break during either meiosis or mitosis, and none of these cases were formed through an intrachromosomal U-type exchange. Murmann et al [11] suggested that the open DNA end of the acentric fragment is stabilized by the formation of an intrachromosomal loop promoted by the presence of sequence with inverted homologies, and the neocentromere formation stabilizes the fragment that is duplicated during an early mitotic event and survives during cell division. Sheth et al [10] suggested that the presence of inv dup(Yp) provides an additional evidence against the U-type exchange mechanism that was proposed by Liehr et al [12] for the formation of inv dup chromosomes.

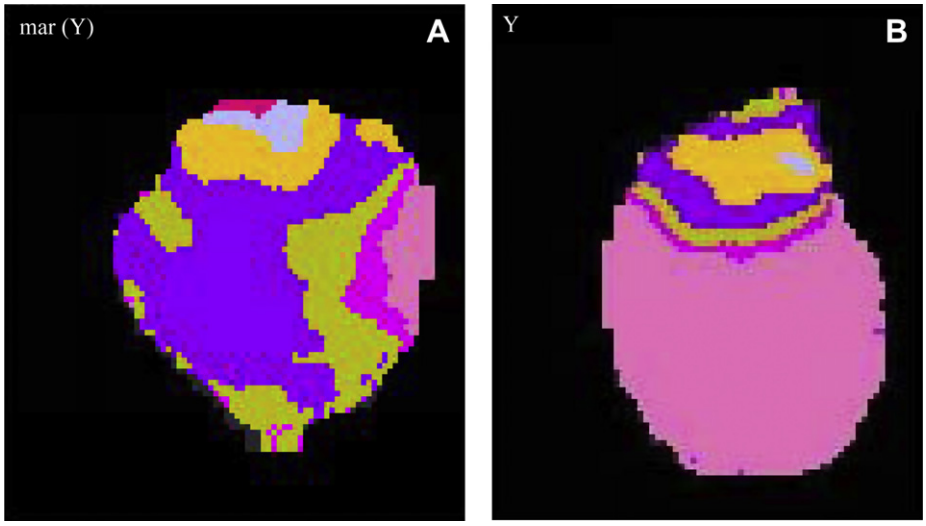

Fig. 3. Multicolor banding analysis shows that the marker (mar) Y chromosome (A) is derived from the short arm and centromere of Y chromosome. (B) A normal Y chromosome.

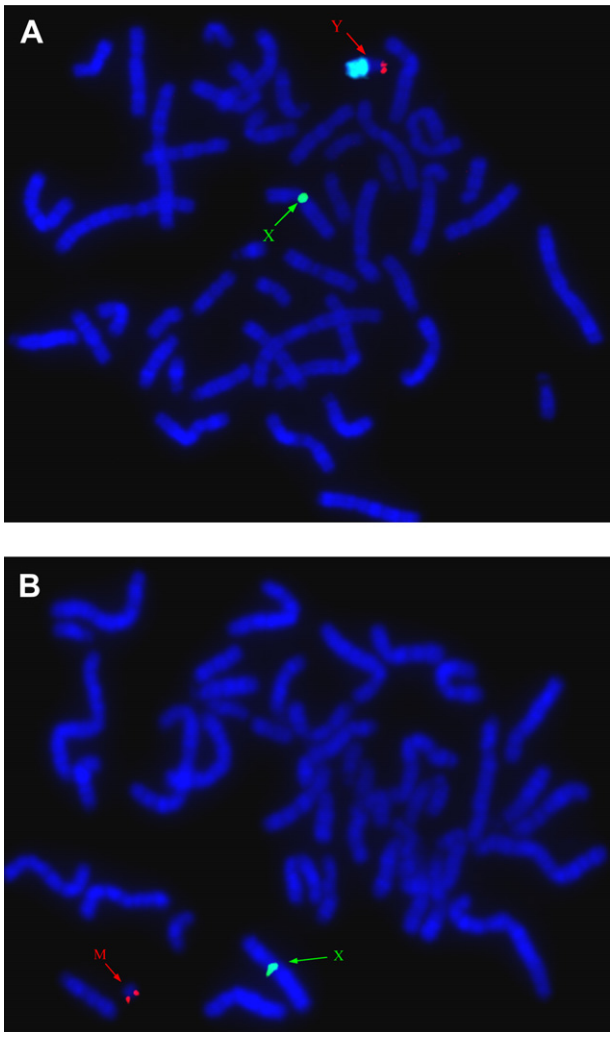

Fig. 4. Fluorescence *in situ* hybridization analysis using CEPX (spectrum green) (Xp11.1-q11.1), *SRY* (spectrum red) (Yq11.31), and *DYZ1* (spectrum blue) (Yq12) probes shows (A) a normal Y chromosome containing both *SRY* and *DYZ1* signals and (B) a marker Y chromosome containing *SRY* signal but no *DYZ1* signal. CEPX = chromosome enumeration probe X; M = marker chromosome; X = X chromosome; Y = Y chromosome.

sMCs with neocentromere formation in Yq have also been described [13–15]. Warburton et al [13] reported a neocentric chromosome of inv dup(Yq)(qter-q11.2::q11.2-qter) consisting of Yq heterochromatin and distal Yq euchromatin but lacking detectable α-satellite DNA. Floridia et al [14] reported *de novo* mos 45,X[8]/46,X,rea(Y)[18] at amniocentesis in a female fetus with cystic hygroma and intrauterine fetal death. The rearranged Y chromosome presented as an sMC and was found to be rea(Y)(qter-q11.2::q11.2-qter) consisting of an inv dup of the long-arm heterochromatin, a small amount of euchromatin, and a neocentromere, but lacking a normal centromere. Assumpção et al [15] reported 46,X,rea(Y) in an 18-year-old woman with primary amenorrhea and hypogonadism. The rearranged Y chromosome presented as an sMC and was shown to be rea(Y)(qter-q11::q11-qter) that lacked Yp and Y-centromere sequences.

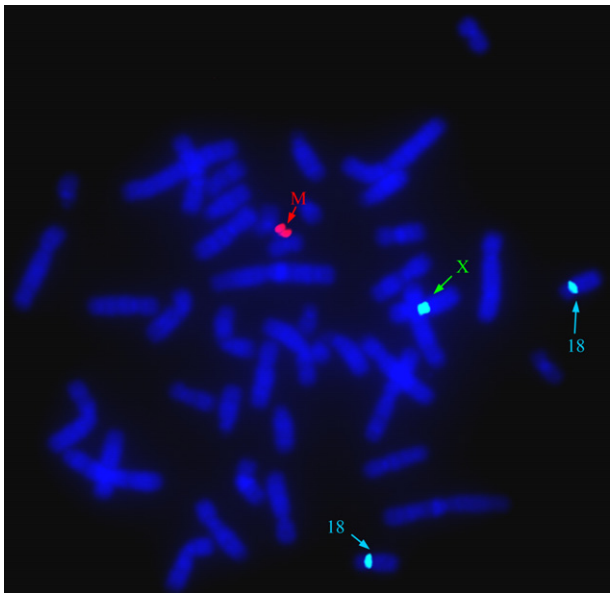

Fig. 5. Fluorescence *in situ* hybridization analysis using CEPX (spectrum green) (Xp11.1-q11.1), CEP18 (spectrum blue) (18p11.1-q11.1), and *DYZ3* (spectrum red) (Yp11.1-q11.1) probes shows that the marker Y chromosome is *DYZ3* positive. M = marker chromosome; X = X chromosome.

A Y chromosome with a primary constriction in the heterochromatic region presenting as an analphoid Y chromosome has been reported [16,17]. Bukvic et al [16] reported an abnormal analphoid Y chromosome presenting as a supernumerary analphoid Y chromosome in a minor cell line (5%) of lymphocytes in association with a normal 46,XY cell line (40%) and a 45,X cell line (55%) in a 29-year-old dysgenetic infertile woman. The analphoid Y chromosome was morphologically identical to the patient's Y chromosome but presented a primary constriction in the heterochromatic region and was analphoid. Tyler-Smith et al [17] additionally reported an analphoid Y chromosome with a fully functional neocentromere transmitted through three generations. In their report, an analphoid Y chromosome with an inactivated normal centromere and a neocentromere in the long-arm heterochromatin was detected at amniocentesis in a 38-year-old woman whose husband also had the same analphoid Y chromosome.

The *SRY* gene [Online Mendelian Inheritance in Man (OMIM) 480000] (Yp11.31) encodes a testis-determining factor and involves a synergistic action on steroidogenic factor-1 (OMIM 184757) (9q33) and SRY-BOX9 (OMIM 608160) (17q24.3-q25.1) to regulate male sex determination [18]. A circular r(Y) results from the fusion of the two broken short and long arms of a Y chromosome. The present case has mosaicism for an *SRY*-positive r(Y) and is expected to be a normal male. However, mosaic Ypter deletion and Yq deletion in the present case raises concern of the effect of partial Yq deletion involving the azoospermia factor (AZF) region (Yq11) on male spermatogenesis [19].

In summary, the present case reports prenatal molecular cytogenetic analysis of an sMC derived from Y chromosome.

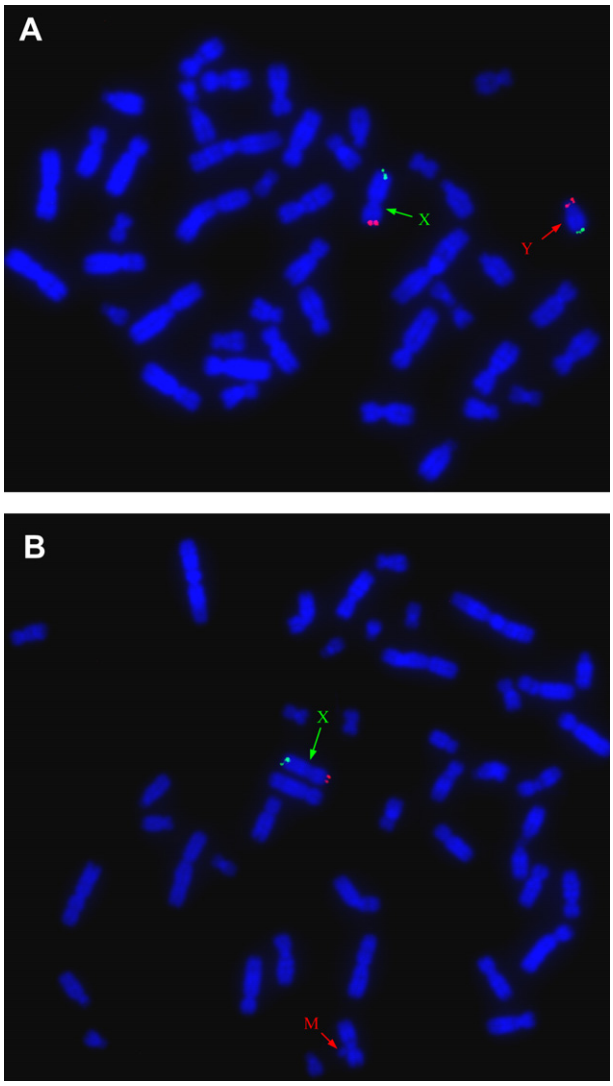

Fig. 6. Fluorescence *in situ* hybridization analysis using Xp/Yp subtelomeric probe (spectrum red) (Xp/Yp pseudoautosomal region subtelomeres) and Xq/Yq subtelomeric probe (spectrum green) (Xq/Yq pseudoautosomal region subtelomeres) shows (A) a normal Y chromosome containing both Yp and Yq signals and (B) a marker Y chromosome containing neither Yp signal nor Yq signal. M = marker chromosome; X = X chromosome; Y = Y chromosome.

The present case demonstrates the usefulness of FISH, SKY, MCB, and aCGH in clinical investigation of prenatally detected sMCs.

Acknowledgments

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