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Short Report

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A Familial Small Supernumerary Marker Chromosome 15 Associated with Cryptic Mosaicism with Two Different Additional Marker Chromosomes Derived de novo from Chromosome 9: Detailed Case Study and Implications for Recurrent Pregnancy Loss

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Keywords
Cryptic mosaicism · Recurrent pregnancy loss · Small supernumerary marker chromosomes

Abstract
We report a case of familial small supernumerary marker chromosome 15 in a phenotypically normal female with 4 recurrent spontaneous abortions and a healthy child. The initial karyotype showed a small, bisatellited, apparently metacentric marker chromosome, 47,XX,+idic(15)(q11.1), maternally inherited. The proband's mother was mosaic for the idic(15)(q11.1) without pregnancy loss. Reexamination of the proband's karyotype revealed cryptic mosaicism for 1 ring and 1 minute chromosome derived de novo from chromosome 9 in 2% of the metaphases. In FISH analysis, the patient's karyotype was mos 47,XX,+idic(15)(q11.1)mat[100]/49,XX,+idic(15)(q11.1)mat,+r(9;9;9),+der(9)dn[2]. The second spontaneous abortion had trisomy 9 (47,XX,+9); the third had mosaic trisomy 9 in 21% of the nuclei and isodicentric chromosome 15 in 36% of the nuclei (mos 48,XN,+9,+idic(15)(q11.1)/47,XN,+9/47,XN,+idic(15)(q11.1)/46,XN). The first and fourth abortions were not cytogenetically studied. The cause of the spontaneous abortions in this patient is likely the cryptic mosaicism for ring and minute chromosomes 9, and gonadal mosaicism is most probable, due to the 2 abortions.

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The aetiology of recurrent spontaneous abortions (RSA) remains unexplained. In many couples, the cause of RSA is never found, probably because many factors participate in the occurrence of repeated miscarriages. Chromosomal anomalies are known to be the single most common cause of spontaneous abortion. More than 50% of spontaneous miscarriages carry chromosomal abnormalities [Lebedev, 2011]. Ninety-five percent of the chromosomal anomalies are numerical, including approximately 60% trisomies and 20% monosomies. Another 15% of spontaneous abortions have ploidy defects, especially triploidy and tetraploidy. In cases of numerical chromosome anomalies in spontaneous abortions, the parental chromosomes are usually normal. The vast majority of conceptus trisomies are known to be maternal in origin; increased maternal age is associated with nondisjunction, and the amount and position of recombination on nondisjoined chromosomes are altered. The role of structural chromosome abnormalities in human pregnancy loss is less than clear, due to the wide variety of chromosomal parts involved in rearrangements, chromosomal breakpoint locations, and affected genes with different functions. Therefore, gathering information about the prevalence of structural chromosome abnormalities in couples with a history of repeated miscarriages is still required to understand their role in the aetiology and pathogenesis of human reproductive pathology, as well as to prevent pregnancy loss via assisted reproductive technologies and preimplantation genetic diagnosis [Franssen et al., 2011; Maithripala et al., 2018].

Small supernumerary marker chromosomes (sSMCs) consist of chromosomal material derived from one or more chromosomes; these structurally abnormal chromosomes cannot be identified or characterized unambiguously by conventional banding cytogenetics alone and are generally equal in size to or smaller than chromosome 20 within the same metaphase spread [Liehr, 2012]. Thus, newer molecular cytogenetic techniques are very important for identifying sSMCs and analysing their structure and origin. sSMCs have been described from all human chromosomes, although most of them are derivatives of acrocentric chromosomes [Dalprà et al., 2005; Manvelyan et al., 2008]. These marker chromosomes are associated with difficulties in conceiving and with repeated pregnancy loss, but the mechanism by which sSMCs influence fertility is not yet understood. It has been suggested that the negative effect of sSMCs on fertility could be due to partial trisomy of some genes related to reproduction, to mechanical effects perturbing meiosis, or to mosaicism level and possible uniparental disomies (UPD) of the chromosomes homologous to the sSMC [Starke et al., 2003; Armanet et al., 2015]. sSMCs are found 2.9 times more often in healthy persons with unexplained infertility than in the general population, and more than 50% of people with fertility problems inherited their marker chromosome from one of their parents, mostly from the mother's side. Studies have shown that men with fertility problems are 7.5 times more likely to have sSMCs than women with fertility problems are [Liehr, 2006; Santos et al., 2007]. In addition to reproductive problems, individuals who are carriers of sSMCs may have a wide range of clinical abnormalities (mental and growth retardation, craniofacial and urogenital abnormalities, abnormal hands and feet, cardiac anomalies, etc.), but 70% of the carriers are clinically normal; thus, it is very important to characterize the content and structure of sSMCs to obtain accurate genotype-phenotype correlations [Liehr, 2014].

Here, we report a familial case of sSMCs 15 and 9 associated with recurrent pregnancy loss.

Clinical Report

A phenotypically normal couple was referred to the Genetic Counselling Unit and Laboratory for Human Genetics after a second spontaneous abortion at 9 weeks of pregnancy for counselling and cytogenetic examination. The female was 41 years old, her husband was 37 years old, and they had a healthy 2-year-old boy. All diagnostic and medical history parameters were assessed and did not reveal any abnormalities. During treatment and counselling, the couple had another 2 miscarriages, at 12 and 15 weeks of gestation, respectively.

Methods

Cytogenetic analyses were performed on peripheral blood cells using standard techniques and GTG-banding. Fluorescence in situ hybridization (FISH) was carried out on metaphase spreads from peripheral blood with DNA probes for the chromosome 9 alpha satellite and 9q21 locus; chromosome 15 centromeric probes for satellite III, D15Z1 (Vysis, Abbott Molecular, UK) and D15Z4 (Kreatech, Leica Biosystems, Germany); and 2 locus-specific DNA probes for the Prader-Willi/Angelman syndrome region, 15q11q12 (*UBE3A*) and 15q11q13 (*SNRPN*) (Kreatech). Long-term culture and paraffin-embedded tissue of the second and third spontaneous abortions were also cytogenetically analysed by FISH using centromere-specific DNA probes for chromosomes 9 and 15.

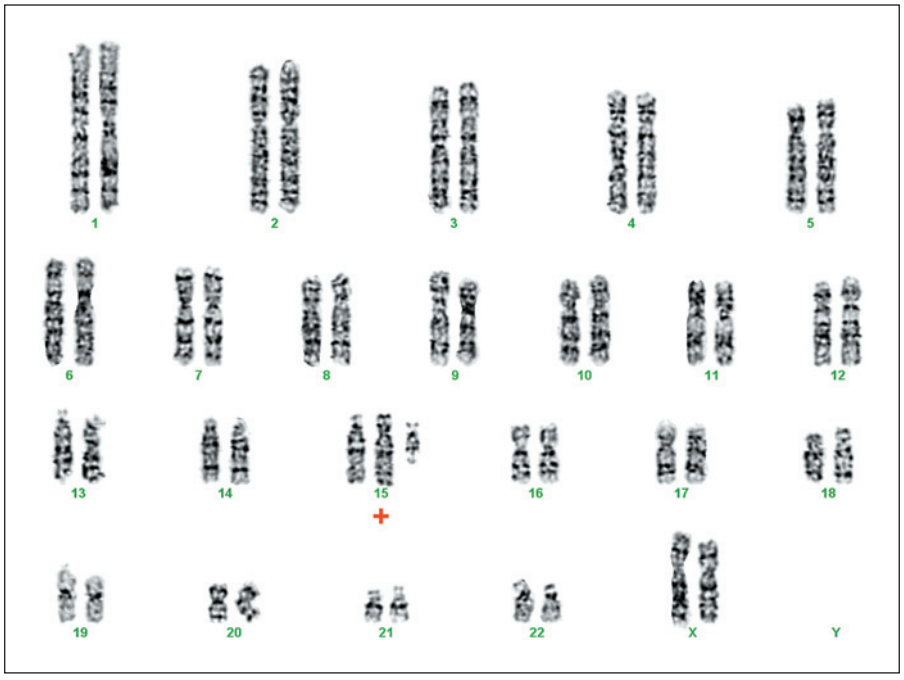

Fig. 1. G-banded karyotype of the patient with marker chromosome 15: 47,XX,+mar.

Results

Analysis of G-banded metaphases from the wife's cultured peripheral lymphocytes showed an extra chromosome that was presumably derived from chromosome 15, yielding the karyotype 47,XX,+mar[100] (Fig. 1). FISH with a centromere-specific probe for chromosome 15 (D15Z4) showed the typical hybridization pattern on the normal chromosomes 15 and 2 signals on the isodicentric marker chromosome 15 in 83% of the cultured lymphocytes (Fig. 2), whereas the remaining cells had only 2 hybridization signals corresponding to a normal karyotype. Probes for the Prader-Willi/Angelman syndrome critical region, including *SNRPN* and *UBE3A*, yielded no hybridization signals on the marker, thereby demonstrating the absence of the region 15q11.2qter. The healthy mother of the proband was also mosaic for idic(15)(q11.1) in 85% of lymphocytes; she had had 2 pregnancies and no history of miscarriages. The healthy husband and the son had normal karyotypes, while the sister of the patient was not cytogenetically examined.

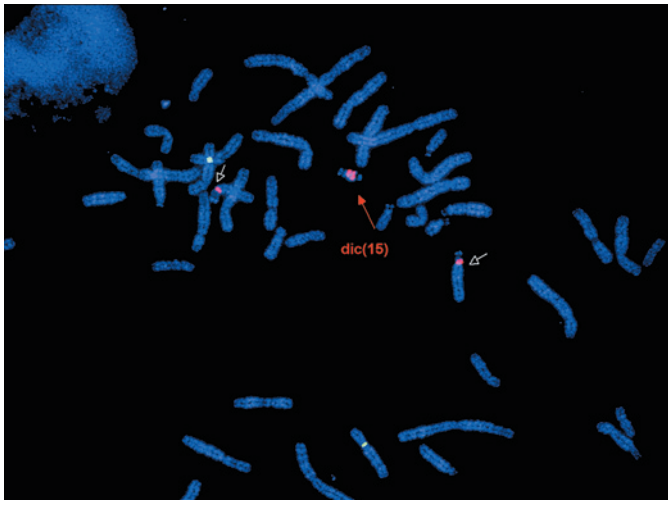

Fig. 2. FISH with centromere-specific DNA probe D15Z4 (red signals) on a metaphase from the patient 47,XX,+idic(15)(q11.1).

After the second spontaneous abortion, which was cytogenetically analysed using long-term culture and showed a cytogenetic result of trisomy 9 in all analysed cells (47,XX,+9[15]) (Fig. 3), additional cytogenetic studies were performed in the proband. FISH analysis with a centromere-specific DNA probe for chromosome 9 and the probe for 9q12 revealed the presence of at least 2 extra sSMCs (Fig. 4) derived from chromosome 9, which confirms a "cryptic mosaicism" involving chromosome 9 in the maternal blood lymphocytes. Molecular cytogenetic analysis showed that both markers consisted of chromosome 9 heterochromatic material. The first was a ring chromosome displaying 4 centromere-

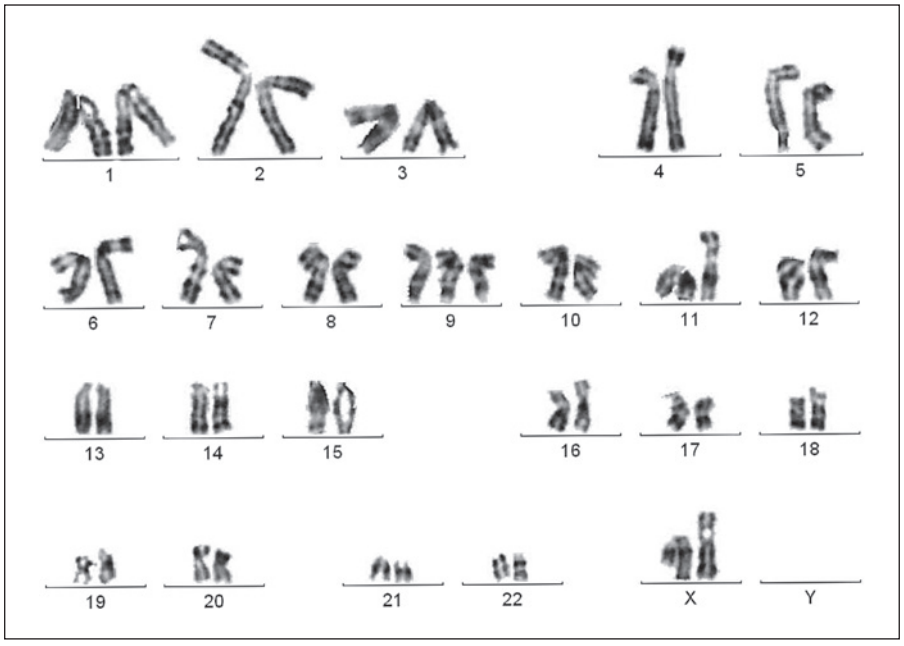

Fig. 3. G-banded karyotype of the second spontaneous abortion: 47,XX,+9.

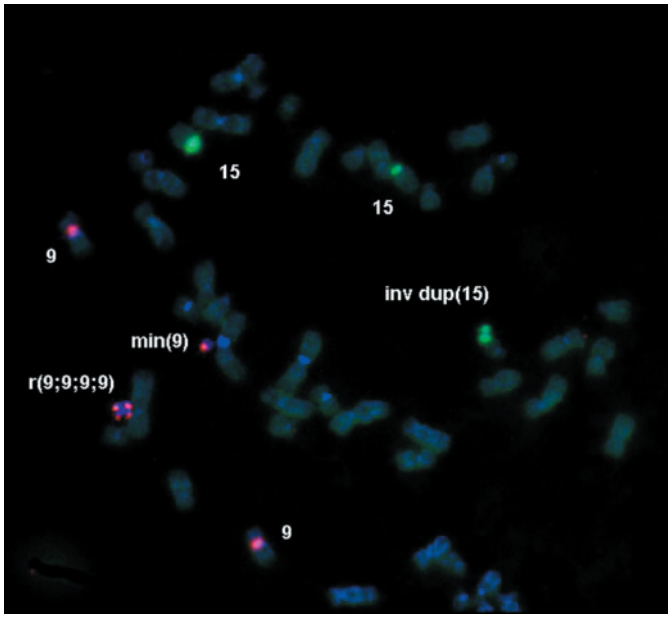

Fig. 4. FISH with centromere-specific DNA probes for chromosomes 9 and 15 on a metaphase from the patient's peripheral blood sample.

specific hybridization signals, r(9)(::p12→q12::p12→q12::p12→q12::p12→q12::), and the second was a minute chromosome, der(9)(:p12→q12:), displaying 1 centromere 9 signal.

During testing and counselling, the couple had another 2 miscarriages. The third spontaneous abortion had mosaic trisomy 9 in 21% of the nuclei and an isodicentric chromosome 15 in 36% of nuclei, mos 48,XN,+9,+idic(15)(q11.1)/47,XN,+9/47,XN,+idic(15)(q11.1)/46,XN. The first and fourth abortions were not cytogenetically studied.

Blood samples from the proband before those new pregnancies showed Epstein-Barr virus reactivation (early antigen positive). All other diagnostic haematological, biochemical, microbiological, and immunological tests, as well as gynaecological and urological examination and medical history parameters, did not reveal any abnormalities.

Discussion

Recurrent miscarriage syndrome is characterized by RSA, and its aetiology is still unexplained. Numerical chromosome anomalies are the most common cause of spontaneous abortion, but this is not the case for repeated miscarriages. As the number of miscarriages increases, the probability of further spontaneous abortions with normal karyotype and the probability to lose the next pregnancy increase as well [Nikitina et al., 2016]. However, if one member of the couple is a carrier of structural chromosome abnormalities (balanced transloca-

tions, inversions, marker chromosomes, etc.), usually without any visible clinical effects, their reproductive function may be affected due to the high probability of generating unbalanced gametes, either numerically or structurally, depending on the nature of the chromosomal abnormality. The heterochromatin excess present in the sSMCs of our patient could disturb correct chromosome pairing, and thus, this excess could influence the generation of unbalanced gametes [Ewers et al., 2010; Armanet et al., 2015]. At the same time, the formation of normal gametes should not be completely excluded, and the birth of a child with a normal karyotype is possible. In accordance with this statement, trisomy of chromosome 9 in spontaneous abortion material was a target of our experimental design in this study, and we found that similar results had been published by other researchers [Santos et al., 2007].

In this couple, we found in the peripheral blood of the wife an sSMC of chromosome 15, idic(15)(q11.1). This sSMC was familial since it was also found in mosaic form in her mother. One can assume that the effect of an sSMC of chromosome 15 on infertility could be due to possible UPD in the progeny. Indeed, Prader-Willi and Angelman syndromes are well-known as results of upd(15)mat and upd(15)pat, respectively. At the same time, there is no evidence from the literature or our previous studies for any selective disadvantage of upd(15) in human embryo development [Nikitina et al., 2004].

The proband's mother was not analysed for structural abnormalities of chromosome 9, while the proband had low-level mosaicism for der(9). It seems likely that the observed derivatives of chromosome 9 in the proband also indicate ring chromosome instability. Indeed, the origin of the 4 centromere-specific regions in ring chromosome 9 may be explained by an odd-numbered series of sister chromatid exchanges during S phase, leading to a doubling in size of the ring chromosome and an increase in centromere number [Pristyazhnyuk and Menzorov, 2018].

Furthermore, we did not find any major or minor clinical anomalies in the proband or her mother. The reproductive history of the proband's mother was unremarkable. During counselling, the couple had another 2 miscarriages. The wife had Epstein-Barr virus reactivation (early antigen positive) in blood samples before the 2 latter pregnancies, which could explain her immune deficiency at that time as well as possible genomic instability [Gruhne et al., 2009; Al-Buhtori et al., 2011].

Conclusion

Although the potential risk of spontaneous abortions caused by sSMCs cannot be defined precisely, marker chromosomes are also factors to be considered when investigating recurrent pregnancy loss. Furthermore, identification of the origin of a marker chromosome may provide additional information for phenotype-karyotype correlations. Further studies, such as molecular analysis to identify the breakpoints, are necessary for investigating phenotype-genotype correlations and assessing the genetic and reproductive risks of sSMCs in patient karyotypes. The cause of the spontaneous abortions in this couple might be the presence of the marker chromosome; trisomy 9 in the second spontaneous abortion and trisomy 9 mosaicism in the third spontaneous abortion are the reasons for suggesting gonadal mosaicism in this woman, but the heterochromatin excess represented by the sSMCs in our patient could disturb correct chromosome pairing, which could influence the generation of unbalanced gametes. Consequently, we recommended genetic counselling before further pregnancies or preimplantation genetic diagnosis to eliminate the transfer of embryos with abnormal karyotypes.

Acknowledgements

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare no conflicts of interest.

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