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Case Report

The First Case of a Small Supernumerary Marker Chromosome 18 in a Klinefelter Fetus: A Case Report

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What's Known

- Small supernumerary marker chromosomes (sSMCs) can rarely be identified prenatally. Often, the origins of sSMCs cannot be clearly determined using common conventional cytogenetic methods.
- To date, only four reports are available regarding the coincidence of Klinefelter syndrome with an additional sSMC.

What's New

- The origin of sSMC is determined using quantitative fluorescent PCR (QF-PCR) and fluorescent in situ hybridization (FISH) techniques
- The first case of prenatal diagnosis of a Klinefelter fetus with an sSMC derived chromosome 18 is presented.

Abstract

Small supernumerary marker chromosomes (sSMCs), or markers, are abnormal chromosomal fragments that can be hereditary or de novo. Despite the importance of sSMCs diagnosis, de novo sSMCs are rarely detected during the prenatal diagnosis process. Usually, prenatally diagnosed de novo sSMCs cannot be correlated with a particular phenotype without knowing their chromosomal origin and content; therefore, molecular cytogenetic techniques are applied to achieve this goal. The present study aimed to characterize an sSMC in a case of Klinefelter syndrome using an in-house microsatellite analysis method and fluorescent in situ hybridization (FISH) technique. Amniotic fluid was collected from a pregnant woman who was considered to have risk factors for trisomy higher than the screening cut-off. Karyotype analysis was followed by the amplification of different microsatellite loci and FISH technique. Karyotype analysis identified a fetus with an extra X chromosome and also an sSMC with unknown identity. Further investigation of the parents showed that the sSMC is de novo. Microsatellite amplification by quantitative fluorescent PCR (QF-PCR) and FISH analysis showed that the sSMC is a derivative of chromosome 18. Eventually, the patient decided to terminate the pregnancy. Here, the first case of the coincidence of sSMC 18 in a Klinefelter fetus is reported.

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Introduction

sSMCs are known as chromosomal fragments or markers which are structurally abnormal.[1] The frequency of an extra structurally abnormal chromosome with unidentifiable origin has been identified in ~0.043% of live births and ~0.072% of prenatal cases[2] and are seven times more common in mentally disabled patients.[2] Approximately 77% of sSMCs are de novo and 23% are hereditary, which can be inherited either from the mother (16%) or the father (7%).[3] Although ~70% of all de novo sSMC carriers are clinically normal,[2] in some cases de novo sSMCs can result in partial trisomy and phenotypic effects.[3] De novo sSMCs were found to be significantly associated with increased maternal age.[4]

Acrocentric chromosomes are the main origin of sSMCs and chromosome 15 is the most frequent origin of de novo cases that contains 50% of acrocentric chromosomes-originated sSMCs.[5] Non-acrocentric derived sSMCs are rare and occur with a frequency of ~15% of all markers and are generally suspected to be small ring chromosomes.[1] Often, the origins of sSMCs cannot be determined clearly using common conventional cytogenetic methods. Therefore, molecular approaches are necessary for definitive characterization. Except for chromosome 15 sSMCs, the karyotype-phenotype correlation is generally unknown and the phenotypic effects can be seen from normal to dysmorphic features and/or delay in developmental processes. It is proven that the phenotype depends on the chromosomal region present in sSMCs, the level of mosaicism, and distribution pattern of the sSMC in different tissues.[6] The chromosomal origins of sSMCs are important in prenatal diagnosis because sSMCs usually cannot be correlated with a particular phenotype without knowing their chromosomal origin and content; therefore, molecular cytogenetic techniques are commonly applied towards achieving this goal.

One of the most common inborn sex chromosomal disorders is Klinefelter syndrome (47,XXY) which was first described in 1942 and has an incidence of 1-2 per 1000 male neonates.[6] One of the most accurate and rapid molecular techniques for detecting aneuploidies is to apply multiplex amplification of microsatellite markers that consists of amplifying polymorphic regions located on the chromosomes of choice to determine the number of copies of those chromosomes present for each cell. Utilization of microsatellite markers provides the possibility of detecting full and partial trisomies with a high accuracy.

To the best of our knowledge, to date, only four reports are available on patients with Klinefelter syndrome and an additional sSMC.[7-10] Herein, the first case of prenatal diagnosis of a Klinefelter fetus with an sSMC derived chromosome 18 is presented.

Case Presentation

A 43-year-old pregnant woman (gravida 4, para 3) underwent fetal first trimester screening test and anomaly scan at 11 weeks and 6 days of gestation. All parameters of the scan were normal and nuchal translucency thickness (NT) was 1.4 mm. Considering the advanced maternal age, biochemical tests of maternal serum were performed. The results showed that the risk for trisomy was higher than the screening cut-off. The patient and her husband were consanguineous and phenotypically normal. Amniocentesis was performed at 12 weeks of gestation at the Comprehensive Medical Genetic Center, Shiraz University of Medical Sciences, Shiraz, Iran. A written informed consent was obtained from the patient for sample collection and subsequent analysis and publication.

Karyotype of the patient showed an extra X chromosome and an sSMC in all 20 metaphase spreads. The determined karyotype was 47,XXY,+mar (figure 1A), which was compatible with apparently Klinefelter syndrome male with an additional marker chromosome. To investigate further, karyotype was performed for both parents on PHA stimulated lymphocyte cultures; both were normal (figures 1B and 1C). By designing an in-house QF-PCR mix, amplification of microsatellite markers on chromosomes 13, 18, 21, X, and Y was performed separately. The QF-PCR mix for chromosome 18 contained four primer pairs to amplify four microsatellite markers on chromosome 18. Furthermore, to determine the relative dosage between chromosomes 18 and 1, a single primer pair was designed for the amplification of one segmental duplication on chromosomes 18 and 1. Sex chromosomes QF-PCR mix contained five primer pairs for the amplification of 5 microsatellite markers on chromosomes X and Y. Additionally, four primer pairs were designed to amplify 4 segmental duplications on chromosomes X, Y, and 3. All forward primers were fluorescently labeled with different fluorochromes (6-FAM, VIC, NED, and PET) (Applied Biosystems, USA).

QF-PCR analysis revealed normal results for chromosomes 21 and 13 but it demonstrated

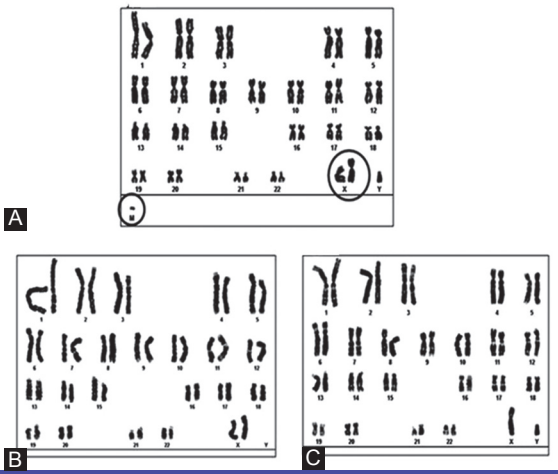

Figure 1: Karyotype results for the fetus and both parents. 1A) Fetus karyotype showing the presence of an extra chromosome X and an sSMC in fetal cells. 1B) Normal karyotype results for the mother. 1C) Normal karyotype results for the father.

the presence of two chromosomes X and one chromosome Y (figure 2A). In addition, 2 markers located on the short arm of chromosome 18 (GATA178F11 and D18S391; locations: 18p11.32 and 18p11.31, respectively) showed trisomic patterns. The other markers on the long arm of chromosome 18 (D18S535, D18S386, and STS18/1; locations: 18q12.3, 18q22.1, and 18q12.1/1p32, respectively) showed normal patterns (figure 2B). This result meant that the fetus is a Klinefelter case and trisomic for the short arm of chromosome 18. Considering amplicon sizes, the origin of sSMC 18 and extra X chromosome was identified as maternal and caused by nondisjunction during maternal meiosis I and II, respectively (figures 3A and 3B).

For a more precise characterization, FISH analysis was performed using POSEIDON centromeric and subtelomeric probes containing chromosome 18 centromere probe, SE18 (D18Z1), and a probe for subtelomeric region of the short arm of chromosome 18 in 40 metaphase spreads (figure 4). According to FISH analysis, the marker chromosome was a derivative of chromosome 18 and contained centromeric region of the short arm of chromosome 18. Based on this finding, the parents decided to terminate the pregnancy.

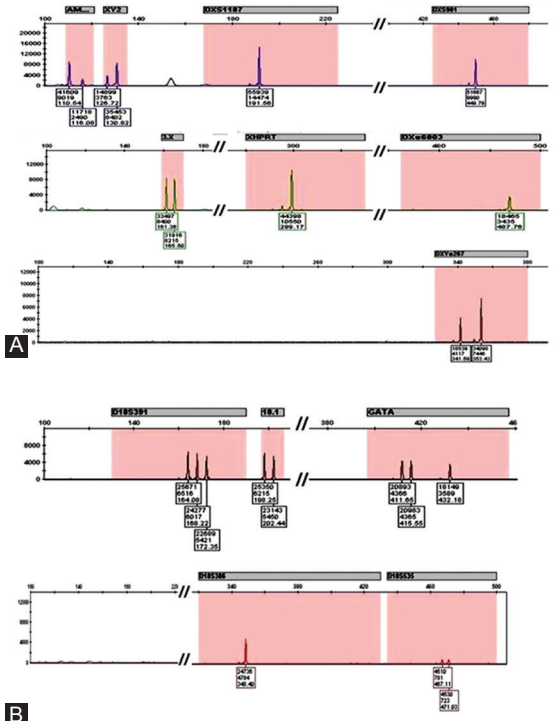

Figure 2: QF-PCR was performed for the fetus and the result is shown by electrophoretogram. 2A) Chromosomes X and Y. AMELXY, XY2, and DXYS267 markers show trisomic diallelic pattern with peak ratio 2:1, 1:2, and 1:2, respectively, confirming the presence of an extra X chromosome. Relative dosage comparison between chromosomes X and 3 (3.X) shows two peaks of identical size, indicating the presence of two chromosomes X and two chromosomes 3. STR markers containing DXS1187, DXS981, and DXS6803 show one peak due to the presence of homozygous alleles. 2B) Electrophoretograms of chromosome 18 multiplex PCR products. D18S391 and GATA markers demonstrate trisomic triallelic pattern and D18S535 marker shows normal diallelic pattern. Relative dosage comparison between chromosomes 18 and 1 shows two peaks of identical size, indicating the presence of two chromosomes 18 and two chromosomes 1. D18S386 marker shows one peak due to the presence of homozygous alleles.

Discussion

A marker chromosome is a chromosome with an abnormal structure which is mainly detected by conventional banding cytogenetics. According to the international system for human cytogenetic nomenclature (ISCN), marker chromosomes are "structurally abnormal chromosomes of unknown origin and commonly found in karyotypes of cancer patients and patients with constitutional genetic disorders" (ISCN 2009).

Approximately 3 million humans in a population of 7 billion are sSMC carriers and sSMCs can originate from any of the human chromosomes.[5]

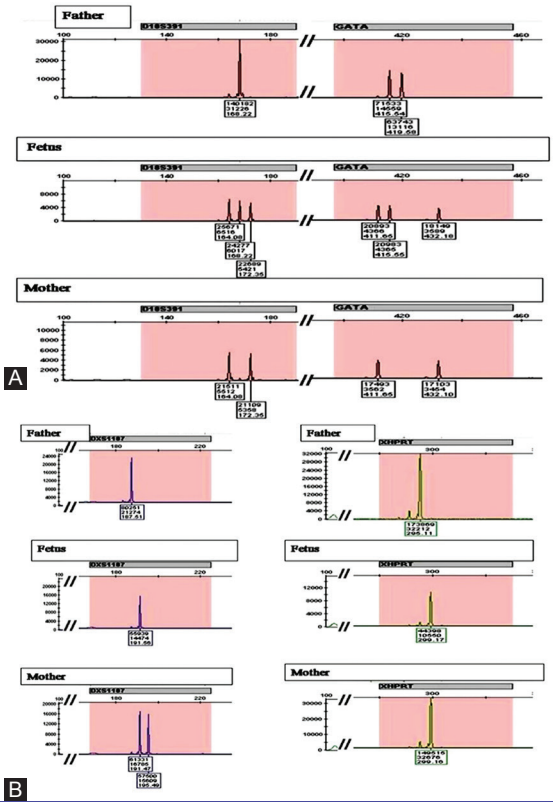

Figure 3: The result of QF-PCR for the father, mother, and fetus shown by electrophoretogram. A) Paternal, maternal, and fetal allelic sizes of some amplified markers on chromosome 18 showed that the origin of sSMC 18 is maternal and nondisjunction occurred during maternal meiosis I. B) Paternal, maternal, and fetal allelic sizes of some amplified markers on chromosome X showed that the origin of the extra X chromosome is maternal and nondisjunction occurred during maternal meiosis II.

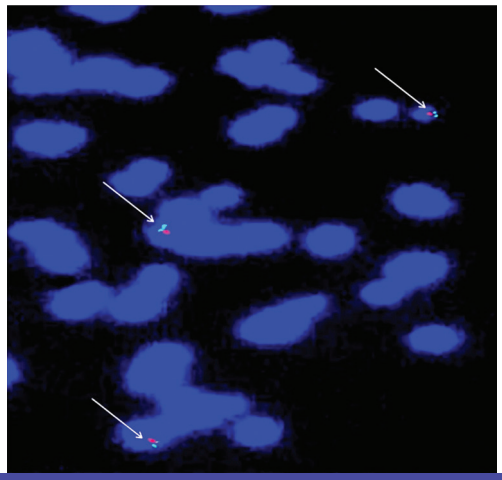

Figure 4: Characterization of sSMC using chromosome 18 centromere (red) and short arm subtelomeric region (green) fluorescent probes. The result demonstrated that the sSMC is a derivative of chromosome 18.

If the same sSMC is present in one of the parents of a fetus, it is a familial sSMC and usually does not lead to a particular phenotype. In the case of a de novo sSMC, phenotypic effects can be present because of extra genetic sequences of the sSMC.[5] sSMCs can additionally be present in a karyotype of 46 normal chromosomes, in a numerically abnormal karyotype (e.g. Klinefelter syndrome or Down syndrome), or in a structurally abnormal but balanced karyotype.[6] Although sSMCs are present in 0.075% of "unselected" prenatal individuals, they can only be detected in 0.044% of postnatal cases. The reduced rate of sSMCs in postnatal cases compared to prenatal can be due to: (i) the effect of maternal age on the need for prenatal diagnosis,[4] (ii) karyotyping, as an invasive prenatal diagnosis method, is carried out in one-third of the cases with a suspected condition, and (iii) the fact that 4.4% of sSMC pregnancies end with stillbirth or spontaneous abortion.[3]

There are a few reports regarding the coincidence of Klinefelter syndrome and sSMCs. For the first time in 1997, Manea et al. presented a Klinefelter case with a marker chromosome in a 2.5-year-old male having developmental delay. That patient was the first case of ring(X) chromosome described in a 47,XXY patient using FISH analysis with an X chromosome paint.[9] In 2005, Liehr et al. presented the second case with 48,XXY,+mar karyotype determined by conventional cytogenetic analysis. Using centromere-specific multicolor FISH (cenM-FISH) and a specific subcentromere-specific (subcenM-FISH) probe, the origin of sSMC was recognized as dic(9)(:p12-->q11.1:q11.1->p11.1:).[8] In 2005, Weimer et al.[10] reported a case of a boy with signs of Klinefelter syndrome, additional mild facial dysmorphic features, and severe speech delay. They identified the boy as a Klinefelter case possessing two different sSMCs. They were characterized by applying FISH analysis and PCR using several Y-chromosome microsatellite markers. One of the two sSMCs was a ring Y chromosome containing the SRY region and the other was the pericentromeric region of chromosome 8.[10] In 2012, Gulten et al. reported another Klinefelter case with marker chromosome originated from chromosome 9.[7] They applied FISH analysis to identify the marker chromosome using the LSI p16 (9p21) SpectrumOrange/CEP 9 SpectrumGreen Probe (Vysis CDKN2A/CEP 9 FISH Probe).[7]

In contrast to all previous reports, we have identified a prenatal case. QF-PCR and karyotype analysis showed an extra chromosome X that was compatible with Klinefelter syndrome. In-house developed QF-PCR revealed trisomy for the short arm of chromosome 18. Since all markers with trisomic pattern belong to the short arm of chromosome 18, in FISH analysis we used probes for the subtelomeric region of the short arm of chromosome 18. For the characterization of the sSMC centromere, chromosome 18 centromere-specific probe (D18Z1) was used. Results from the FISH analysis was in agreement with the QF-PCR outcome and confirmed that sSMC is a derivative of chromosome 18. Since the origin of extra X chromosome and sSMC was maternal, the role of advanced maternal age in chromosomal aneuploidies was reconfirmed.

Conclusion

The first case of the coincidence of Klinefelter syndrome with sSMC derived chromosome 18 is reported. The usefulness of QF-PCR and FISH techniques for the characterization of sSMCs content is demonstrated.

Acknowledgment

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Conflict of Interest: None declared.

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