Prenatal and Postnatal Clinical Spectrum of a Mosaic Small Supernumerary Marker Chromosome 22

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Abstract: An amniocentesis was performed because of the advanced maternal age at 18 weeks of gestation, and cytogenetic analyses revelaed a de novo mosaic small supernumerary marker chromosome (sSMC), as parental karotypes were normal. Fluorescence in situ hybridization (FISH) analyses with chromosome specific whole chromosome painting, locus, and alphoid satellite DNA probes were ended with the diagnosis of mosaic sSMC of chromosome 22. The result was explained to the family and genetic counselling was given together with the absence of fetal ultrasound findings. The baby was delivered at term by caesarean section. The female neonate with mosaic sSMC did not show any apparent dysmorphic features at birth. No growth and psychomotor retardation were observed at her natal period follow-up. At one year age, she had some mild dysmorphic findings such as high frontal hairline, frontal bossing, thin eyebrows, hypertelorism, flattened tip of nose and prominent philtrum. The reason of the absence of major abnormalities could be related with the presence of heterochromatin region of chromosome 22 rather than the gene rich parts. Finally, this report emphasizes the importance of acting in an analytical algorithm based on chromosomal origin, parental lineage, size and mosaic or non-mosaic status of sSMCs in the prenatal diagnosis, and using FISH technique and postnatal confirmation of the sSMC.

Keywords: Amniocentesis, Chromosome 22, Cytogenetic analysis, Fluorescence in situ hybridization, Prenatal diagnosis, Small supernumerary marker chromosome.

INTRODUCTION

Small supernumerary marker chromosomes (sSMCs) are defined as a structurally abnormal chromosomes that cannot be characterized by conventional cytogenetic analysis, and has a size equal to or smaller than a chromosome 20. [1-3]. The incidence of sSMCs is estimated at 0.14 to 0.72 per 1,000 live birth [4]. About 70% of sSMCs are de novo [5, 6] and about 70% of sSMCs are originated from acrocentric chromosomes [2, 5, 7]. In approximately 28% of cases with these markers, an abnormal phenotype is observed usually excluding the derived from the acrocentric chromosomes [4]. Almost 9% of all sSMCs are originates from chromosome 22 [8]. Prenatal and postnatal diagnosis of sSMCs and evaluation in terms of the genes it contains gives rise to difficulties in genetic counseling, and identification of the aberrant chromosome structure requires the use of molecular cytogenetic techniques such as Fluorescence in situ Hybridization (FISH) [5, 9, 10]. Herein, we report the prenatal diagnosis and molecular cytogenetic characterization of mosaic sSMC derived from chromosome 22 and its postnatal confirmation in a infant with mild dysmorphic features.

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CLINICAL REPORT

A 40-year old, gravid a 4, para 2, living 2, dilation and curettage (D&C) 1, woman underwent amniocentesis at 18 weeks of gestation because of her advanced maternal age. She and her husband were non consanguineous and healthy, and there was no family history of congenital malformations. Cultivation of amniocytes and peripheral blood lymphocytes, harvesting procedures, and G-banding followed standard protocols. Karyotypes were interpreted according to the ISCN 2013 [11]. Cytogenetic analysis of the amniotic fluid culture showed mosaic marker chromosome (mos 46,XX/47,XX+mar) in 37 of 189 (19,5%) colonies found in three of the independent in situ tissue cultures (Figure **1**). Constitutive heterochromatin (C) banding and nucleolar organizing region-stain (NOR) were performed as described by Salamanca & Armendares [12] and Archidiacono *et al*. [13], respectively. The sSMC was C and NOR band positive. Simultaneously performed cytogenetic analysis of the phenotypically normal parents revealed normal constitutional karyotypes. FISH technique on metaphase and interphase cells was performed using standard protocols [14, 15]. FISH analysis was firstly performed to centromeric region using alphoid satellite DNA probes (centromeric region) for acrocentric chromosomes in order of 15 (Catalog no: LPE015R/G, Cytocell, Cambridge) and 14/22 (Catalog no: LPE014R/G, Cytocell, Cambridge) and the marker

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chromosome was determined to originate from chromosome 22. Later various FISH applications were performed in order to determine the composition of the marker derived from chromosome 22. For this purpose, locus-specific probes (DiGeorge syndrome probe TUPLE1 (HIRA) on 22q11.21 and ARSA as its control probe on 22q13.33 (Catalog no: LPU 004, Cytocell, Cambridge); additionally BCR on 22q11.23, and subtelomeric probes for chromosome 22 (22qter) (Vysis, Downers Grove, IL) were used after the whole chromosome painting (WCP) probe for chromosome 22, respectively (Figure **2**). At the end of all this FISH applications, final karyotype was reported as mos 46,XX/47,XX,+mar.ish mar(22)(p11.1q11.2) (D22Z1+,WCP22+,HIRA+,BCR-,22qter-,ARSA-)dn.

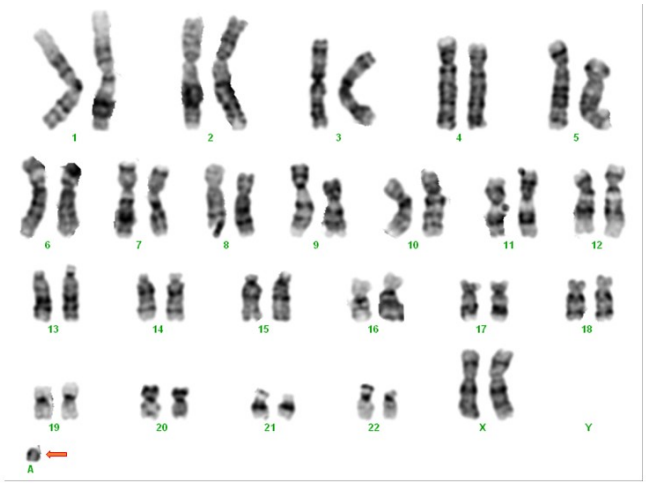

Figure 1: The karyotype of the amniotic fluid culture in GTG 450–550 band levels. The arrow indicate the small supernumerary marker chromosome.

Where upon, a new amniocentesis procedure with high-resolution detailed fetal ultrasonography (USG) were recommended to fetus. Due to absence of any abnormality on fetal USG, the family did not want to perform a second invasive method. The parents notified the reliable and fast detecting of the prenatal sSMC22 by the help of FISH and cytogenetic techniques, and also they were given the accurate unbiased genetic counseling on the possible clinical effects of sSMC22. The postnatal cytogenetic analyses confirmation, and phenotypic evaluation of the baby were recommended, as the family was decided to continue the pregnancy.

She was born at term by caesarean section. Her birth weight was 3,600 g (75 percentile), length and occipitofrontal circumference in birth were unmeasured. The proband was brought to the medical examination by her family at postnatal 10th month. The weight, height and occipitofrontal circumference were consistent with her age. Her head control and sit without support were normal. Psychomotor development was also normal. In the craniofacial examination, she had mild dysmorphic findings such as high frontal hairline, frontal bossing, thin eyebrows, hypertelorism, flattened tip of nose and prominent philtrum. Her trunk, extremities and external genitals were natural. Cytogenetic analysis of peripheral venous blood for the purpose of postnatal karyotype confirmation, revealed 42% marker chromosome.

DISCUSSION

A small part of any human chromosome can be additionally exists on metaphase spreads as sSMC [2].

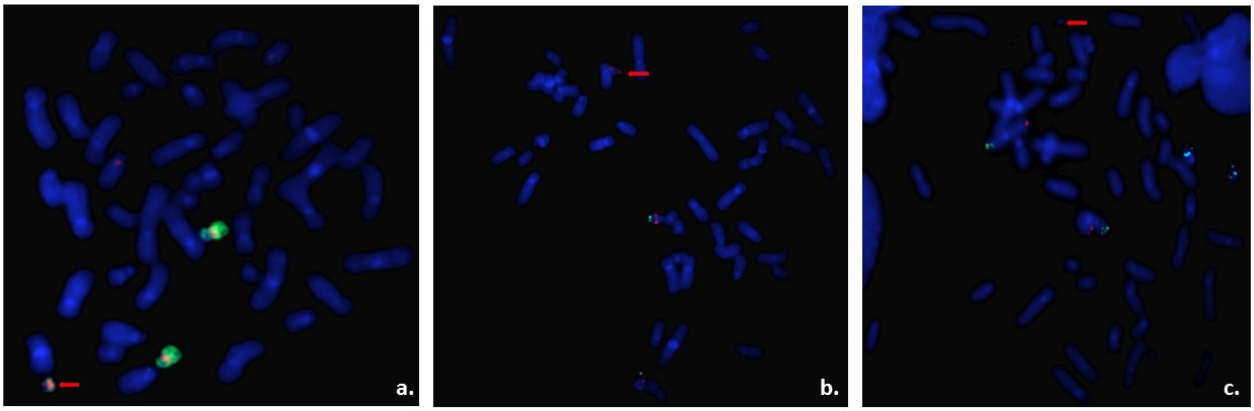

Figure 2: The FISH studies of the amniotic fluid culture, the arrows indicate the sSMC. **a**. Alphoid satellite DNA probes (centromeric region) for chromosome 14/22 (red signals) and whole chromosome painting (WCP) probe for chromosome 22 (green signals). Small supernumerary marker chromosome (sSMC) has cep14/22 and WCP22; **b**. DiGeorge Syndrome probe TUPLE1 (HIRA) (22q11.2) (red signals) and ARSA (22q13.3) as its control probe (green signals). sSMC22 has TUPLE1; **c**. In this FISH application, subtelomeric probe for chromosome 3 and its control probe 22qter and locus specific probe for BCR (22q11) were used. sSMC22 did not show any signal except DAPI (4′,6-diamidino-2-phenylindole: a fluorescent stain that binds strongly to adenine–thymine rich regions in DNA).

To detect the origin, and clinic manifestations of sSCMs are extremely difficult and stressful in prenatal period, because of uncertain phenotype, time limitation for decide on the fate of pregnancy, and unknown genetic content. Despite all these negatives, FISH technique used together with karyotyping, have value to display the content of sSCMs. Moreover, FISH technique is more informative than microarray technique in the presence of low mosaicism, and in the presence of heterochromatin regions [16].

The clinical effect of an sSMC is varies according to 1) euchromatic DNA content, 2) mosaicism degrees, and/or 3) uniparental disomy (UPD) of the relevant chromosome, and outcome is quite difficult to predict because of the heterogene clinics [17]. The difficulty coefficient of this condition increases by several times if the sSMC is determined especially in the prenatal period. Therefore, it is important that the sSMC is evaluated by an experienced team. Herein, the presented case resolved in prenatal period during the amniocentesis analysis quickly and reliably, and the results were announced the family quickly with an accurate genetic counseling.

Some chromosomal regions in the genome are more susceptible to rearrangements; the 22q11 which have breakpoint regions harboring a similar low-copy repeat (LCR) stated as LCR22, is also one of these regions. The rearrengements in the 22q11 region on the homologous recombination between LCR22s during meiosis can cause to some syndromes such as DiGeorge syndrome/velocardiofacial syndrome (DGS/VCFS, OMIM *600594), Cat Eye Syndrome (CES, OMIM #115470) and t(11;22)der(22) syndrome (Emanuel Syndrome, ES, OMIM #609029) [5]. Small SMC22 identified during prenatal diagnosis always requires extra attention because trisomy or tetrasomy of the 22q11 may exhibit a variable phenotype depending on the coverage of CES region candidate genes [9]. The present fetus searched for the 22q11.23 region via FISH and no additional copy of fluorescence signal was observed on this region. Seventy percent of the sSMC22 carriers are clinically normal [2]. In addition, cytogenetic mosaicism significantly influences the clinical outcome of patients with a sSMC [18]. To date, a large number of patients having der(22)(pter→q11.21) which is outside the CES critical region (22q11.2→q13) without clinical findings have been reported [5]. It is known that bisatellited isodicentric marker chromosome including critical region located in the most proximal 2-2.5 Mb of 22q11 is caused CES [16]. Although the sSMC22 and CES patients share a similar region on chromosome 22q11.21, the patients with familial partial trisomy of the proximal 22q have 3 copies for CES critic region, and the CES patients posses 4 copies extra dosage for CES critic region [4, 19]. Therefore, it is important to accurately assess the coverage of sSMC22. Our case has been identified as mosaic trisomic for genes outside the CES critical region by FISH technique, ie sSMC22 was positive for TUPLE1 at 22q11, while for BCR at 22q11.23 and ARSA at 22q13.33 were negative. The family decided to continue pregnancy after the our objective genetic counseling. In the follow-up of the proband after birth, in addition to her mild dysmorphic findings the absence of any anomaly, and her normal psychomotor development confirms our approach.

This report emphasizes the importance of acting in an analytical algorithm based on chromosomal origin, parental lineage, size, and mosaic or non-mosaic status and FISH of sSMCs. The identification of the origin of an SMC provides additional data for genotype–phenotype correlation.

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