Tetrasomy 9p Mosaicism Associated with a Normal Phenotype in Two Cases


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Mosaicism · Normal phenotype · Tetrasomy 9p

Abstract
Tetrasomy 9p is a rare chromosomal syndrome and about 30% of known cases exhibit mosaicism. Approximately 50% of the reported cases with tetrasomy 9p mosaicism show a characteristic facial appearance, growth failure, and developmental delay. However, 3 patients with mosaicism for isochromosome 9p and a normal phenotype have also been reported. We report 2 additional cases of clinically normal young females with tetrasomy 9p mosaicism, one of whom also exhibited X chromosome aneuploidy mosaicism leading to an overall of 6 different cell lines. STR analysis performed on this complex mosaic case indicated that the extra isochromosome was of maternal origin while the X chromosome aneuploidy was of paternal origin, indicating a postzygotic event.

Small supernumerary marker chromosomes (sSMCs) are reported in 0.044% of newborn infants and in 0.125% of subfertile individuals [Liehr and Weise, 2007]. sSMCs are structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional cytogenetics alone, and they are generally equal in size or smaller than chromosome 20 of the metaphase spread [Liehr et al., 2004]. To date, only one report is available for a triple-X syndrome patient with an additional sSMC [Lee-Jones et al., 2004], and only 3 cases have been reported so far with mosaic tetrasomy 9p that present no clinical symptoms [Sait and Wetzler, 2003; McAuliffe et al., 2005; Baronchelli et al., 2011].

In the present study, we report 2 patients with tetrasomy 9p mosaicism and an apparently normal phenotype. The first individual was cytogenetically studied because of a de novo inversion in a chromosome 7, observed in a previous pregnancy. The second proband was referred for cytogenetic studies as part of in vitro fertilization (IVF) pre-testing due to her husband’s azoospermia. The results of the molecular, clinical, and cytogenetic findings are presented and compared to reports previously published.
Case Reports

Case 1
A 20-year-old female was studied cytogenetically due to a previous pregnancy with a de novo pericentric inversion in chromosome 7. The patient presented no dysmorphic features and/or mental abnormalities, and there was no family history of miscarriages and/or genetic abnormalities. Peripheral blood and buccal mucosa were available for cytogenetic studies.

Case 2
A 28-year-old female, the second child of healthy non-consanguineous parents, was studied cytogenetically before starting IVF treatment due to her husband’s azoospermia. The family history was unremarkable. The patient had a height of 169 cm, head circumference of 55 cm, weight 63 kg, had normally developed genitalia, normal menstrual cycle, and an average mental condition. Endocrinological studies revealed no abnormal values (PRL 23.3 ng/ml, FSH 9.7 mIU/ml, LH 3.3 mIU/ml, E2 74.7 pg/ml, PRG 0.41 ng/ml, 17-OH PRG 0.53 ng/ml, and DHEA 946.4 ng/ml).

Methods and Results

Metaphase chromosome preparations were obtained from PHA-stimulated lymphocyte cultures according to standard procedures [Verma and Babu, 1998].

In case 1, the cytogenetic analysis of stimulated blood cells revealed a non-mosaic karyotype of 47,XX,+mar. Multiplex-fluorescence in situ hybridization (M-FISH) [Speicher et al., 1996] showed that the sSMC was a derivative of chromosome 9. Application of a centromeric probe for chromosome 9 (cep 9) in combination with a subtelomeric probe for the short arm of chromosome 9 (9pter) identified the sSMC as an i(9)(p10) (fig. 1a). However, in buccal mucosa, interphase-FISH, using a centromeric probe for chromosome 9, confirmed the presence of the sSMC only in 65% of the examined cells. This finding in association with the normal clinical phenotype of the patient indicates that it is possible that most of the tissues of the patient present a mosaicism for isochromosome 9p rather than a full tetrasomy 9p. According to ISCN [2009], the karyotype was mos 47,XX,+i(9)(p10)/46,XX. Follow-up cytogenetic studies of the patient’s parents were not possible.

In case 2, a routine cytogenetic analysis on peripheral blood revealed a mosaic karyotype mos 48,XXX,+mar[14]/47,XX,+mar[14]/49,XXX,+mar[4]/47,XXX[2]/46,X,+mar[2]/46,XX[4]. Parental chromosome analysis revealed normal karyotypes. Application of an alpha-satellite-specific probe for chromosome 9 (cep 9) together with a microdissection-derived probe (midi36) for the pericentric region of chromosome 9 (9p12/9q13-21.1) and a whole chromosome painting (wcp) probe identified the sSMC as an i(9)(p10) (fig. 1b). Accordingly, the karyotype was designated as mos 48,XXX,+i(9)(p10)[14]/47,XX,+i(9)(p10)[14]/49,XXX,+i(9)(p10)[4]/47,XXX[2]/46,X,+i(9)(p10)[2]/46,XX[4]dn.

DNA was extracted from blood samples using the NucleoSpin blood extraction kit (Macherey-Nagel, Düren, Germany). Uniparental disomy (UPD) of the normal chromosomes 9 was excluded by means of parent-to-patient segregation analysis using a panel of 8 short tandem repeat (STR) markers located along the length of chromosome 9 (D9S103, D9S117, D9S199, D9S194, D9S195, D9S109, D9S193, D9S200). A set of 4 STR markers was also used for the determination of the origin of the X chromosome aneuploidy (DXS990, DXS987, DXS8091, DXS1047). Quantitative fluorescence (QF) PCR was performed to amplify the repeat sequences at the above polymorphic loci, and the primer sequences were probed with fluorescent labels as described elsewhere [Mann et al., 1996].

Fig. 1. a Partial karyogram of case 1 showing both normal chromosomes 9 and the isochromosome 9p in inverted DAPI-banding and after FISH. An alpha-satellite-specific probe for chromosome 9 (cep 9) and a subtelomeric probe for 9pter (subtel 9pter) were applied. b Both normal chromosomes 9 and the isochromosome 9p found in case 2 after FISH using an alpha-satellite-specific probe for chromosome 9 (cep 9) together with a microdissection-derived probe (midi36) for the pericentric region of chromosome 9 (9p12/9q13-21.1) and a whole chromosome painting (wcp) probe.
The fluorescent QF-PCR products were analyzed by capillary electrophoresis on an automated DNA sequencer (ABI 3100, Applied Biosystems, Carlsbad, Calif., USA). STR analysis indicated that the extra isochromosome was of maternal origin, while the X chromosome aneuploidy observed in case 2 was of paternal origin (all extra copies of chromosome X).

STR analysis was not performed in case 1 as no parental DNA material was available.

Discussion

Tetrasomy 9p is a rare syndrome, and about 30% of known cases exhibit chromosome mosaicism [Stumm et al., 1999]. Reports in the literature of cases with tetrasomy 9p, about 50 including mosaic and non-mosaic cases [Liehr, 2011], showed characteristic facial appearance with hypertelorism (72%), broad nasal root or bulbous/beaked nose (69%), cleft lip or palate (78%), ear anomalies (88%), micrognathia (59%), developmental delay (94%), central nervous system anomaly (89%), limb defects (88%), postnatal growth failure (71%), congenital heart disease (62%), small gestational age (57%), renal anomalies (57%), wide sutures/large fontanelle (56%), and short neck/excess nuchal skin (53%) [Dhandha et al., 2002].

There are 3 patients depicted in the literature with mosaicism for isochromosome 9p and a normal phenotype [Sait and Wetzler, 2003; McAuliffe et al., 2005; Baronchelli et al., 2011] (table 1). Sait and Wetzler [2003] described a healthy 41-year-old male with mosaicism of isochromosome 9p who was referred for cytogenetic analysis because of skin lesions; the only abnormality found was hypereosinophilia in the bone marrow and peripheral blood film. McAuliffe et al. [2005] reported a 37-year-old male patient with isochromosome 9p mosaicism with oligospermia who had fathered 2 normal children, and Baronchelli et al. [2011] found an i(9p) in 72% of peripheral blood cells studied cytogenetically in an adult female with premature ovarian failure.

In the 2 additional cases reported here, the chromosomal imbalance of chromosome 9 was not associated with any prominent phenotypic abnormality in the apparently healthy 20- and 28-year-old females. It has been proposed that the degree of phenotypic involvement can be associated with the degree of mosaicism, the size of the isochromosome involved, and the extent of tissue involvement [Grass et al., 1993].

Although a correlation between the level of mosaicism and phenotypic abnormalities has been described, there was no such evidence in our 2 cases. Interestingly, similar findings have been reported for other sSMC cases usually known to have an adverse prognosis but instead presented a mild phenotype, such as additional isochromosome 18p [Kim et al., 2009], inv dup(15)(q13) [Bonati et al., 2005; Loitzsch and Bartsch, 2006], inv dup(22)(q11.21).

| Table 1. Cytogenetic findings and clinical data in 4 mosaic cases with a supernumerary i(9p) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Case 1 | Case 2 | Case 1 | Case 2 |
| Age, years | 37 | 41 | adult | 20 | 28 |
| Sex | male | male | female | female | female |
| Phenotype | normal | normal | normal | normal | normal |
| Reason for investigation | oligospermia | hypereosinophilia in bone marrow and peripheral blood film/skin lesions | premature ovarian failure | previous pregnancy inv(7) | IVF |
| Karyotype GTG-banding | 47,XY,+i(9)(p10)[4]/46,XY[16] | 47,XY,+i(9)(p10)[?100%] | 47,XX,+i(9)(p10)[100%]; in buccal mucosa marker only in 65% of cells | mos 48,XXX,+i(9)(p10)[14]/47,XX,+i(9)(p10)[14]/49,XXX,+i(9)(p10)[4]/47,XXX[2]/46,X,+i(9)(p10)[2]/46,XX[4]dn |
| Origin | n.a. | n.a. | n.a. | n.a. | de novo |
| FISH method | cep 9; subtel 9p | n.a. | n.a. | M-FISH; cep 9; subtel 9p | cep 9; subtel9p |
| Identified sSMC | i(9)(p10) | i(9)(p10) | i(9)(p10) | i(9)(p10) |
| Studied material | PBL, skin | PBL | PBL | PBL, buccal mucosa | PBL |
| PBL = Peripheral blood lymphocytes. |
leading generally to cat eye syndrome [Lin et al., 2006], or even isochromosome 12p leading to Pallister-Killian syndrome [Genevieve et al., 2003]. In our case 1, no mosaicism was evident after studying blood lymphocytes; it became obvious only after interphase-FISH of the buccal mucosa. Still, only a few tissues were studied in both of our cases.

The supernumerary isochromosome 9p in case 2 was a de novo finding as in all of the so far described cases [Dutly et al., 1998; Eggermann et al., 1998; Wyandt et al., 2000] and of maternal origin. It seems that errors in maternal meiosis may be responsible for the origin of the isochromosome and that non-disjunction during meiosis II could be followed by rearrangements leading to duplication of the short arm and loss of the long arm in the majority of cases [Dutly et al., 1998].

For sSMCs in general, the predominant mechanism of origin has been shown to be ring chromosome formation by centromere misdivision, the so-called McClintock mechanism [Baldwin et al., 2008].

Trisomy X occurs from a non-disjunction event in which the X chromosomes fail to properly separate during cell division, either during gametogenesis or after conception [May et al., 1990]. Studies made to determine the parental origin of the additional X chromosome demonstrated that in 58–63% of cases the extra X chromosome derived from maternal meiosis I errors, in 16–17% from maternal meiosis II errors, and in 18–20% from post-zygotic non-disjunction [Hall et al., 2006; Hassold et al., 2007]. One study [Wallerstein et al., 2004] with mos- saic trisomy X (such as 45,X/47,XXX) suggested that cases of mosaicism may result from a post-zygotic non-disjunction event as could be the cause in our case 2. This case presented with a normal stature, while women with a mosaic karyotype of 45,X/47,XXX generally develop a short stature [Syber and McCauley, 2004]. The severity of the short stature has been correlated with the distribution of cell lines in 47,XXX/45,X/46,XX mosaicism [Partsch et al., 1994].

Mosaicism for tetrasomy 9p is a challenging issue in terms of prenatal diagnosis and genetic counseling as the abnormality may not be detectable in the amniotic fluid and fetal ultrasound assessment can be normal throughout pregnancy. In one reported case, amniocentesis due to advanced maternal age showed a normal fetal karyotype. However, further cytogenetic analysis due to postnatal developmental delay revealed mosaic tetrasomy 9p in blood and skin cells [Eggermann et al., 1998].

Our 2 cases of healthy females can be regarded as representing the one end of the spectrum of karyotype-phenotype correlation in chromosomal aneuploidies [Avramopoulos et al., 1997]. In most such cases, however, tissue-specific mosaicism has not been fully investigated.

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Tetrasomy 9p Mosaicism with a Normal Phenotype


Notes Added in Proof

A more intense literature search revealed four (4) additional i(9p) cases with normal phenotype. For more details visit: http://www.fish.uniklinikum-jena.de/sSMC/sSMC+by+chromosome/sSMC+9.html#i9p).