Characterization of 23 small supernumerary marker chromosomes detected at pre-natal diagnosis: The value of fluorescence *in situ* hybridization

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Abstract. Small supernumerary marker chromosomes (sSMCs) cannot be identified or characterized unambiguously by conventional cytogenetic banding techniques. Until recently, the large variety of marker chromosomes, as well as the limitations in their identification, have presented a diagnostic problem. In order to determine the origin of sSMCs, we used a variety of fluorescence *in situ* hybridization (FISH) methods, including centromere-specific multicolor FISH, acrocentric specific multicolor FISH, subcentromere-specific multicolor FISH and multicolor FISH with whole chromosome paint probes. Moreover, uniparental disomy testing was in all cases attempted. From a total of 28,000 pre-natal samples from four diagnostic genetics laboratories in Greece, 23 (0.082%) supernumerary marker chromosomes were detected. The mean maternal age was 36.2 years (range 27-43) and the mean gestational age at which amniocentesis was performed was 18.5 weeks (range 16-23). Eighteen markers were *de novo* and 5 markers were inherited. Molecular cytogenetic methods were applied to determine the chromosomal origin and composition of the sSMC. In total, 17 markers were derived from acrocentric chromosomes (14, 15, 21 and 22) and 6 markers were non-acrocentric, derived from chromosomes 9, 16, 18, 20 and Y. Uniparental disomy was not detected in any of the cases studied. With regard to pregnancy outcome, 13 pregnancies resulted in normal healthy neonates, while 10 pregnancies were terminated due to ultrasound abnormalities. A total of 23 marker chromosomes from 28,000 pre-natal samples (0.082%) were identified. Molecular cytogenetic techniques provided valuable information on the chromosomal origin and composition of all the sSMCs. Especially in cases with normal ultrasound, the FISH results rendered genetic counseling possible in a category of cases previously considered a diagnostic problem. Abnormal outcome was observed in 10 cases (43,5%), 7 of which showed abnormal ultrasound findings. New technologies, such as array-comparative genomic hybridization, should be used in future genotype-phenotype correlation studies, although the high mosaicism rate poses a problem.

Introduction

Small supernumerary marker chromosomes (sSMCs) are structurally abnormal chromosomes, equal in size or smaller than chromosome 20, which cannot be identified or characterized unambiguously by conventional cytogenetic banding techniques (1). These chromosomes are detected in 0.04% of newborn children, whereas in developmentally retarded patients the rate is 0.22% (1-3). sSMCs are also present in 0.08% of unselected pre-natal cases and in 0.20% of pre-natal cases with ultrasound abnormalities (2). The large variety of sSMCs, as well as the limitations in their cytogenetic identification, have presented a diagnostic problem in their interpretation. In general, the risk for an abnormal phenotype is approximately 13%, varying from 7% when *de novo* sSMCs derived from chromosomes 13, 14, 21 and 22 are encountered, to 28% for non-acrocentric

chromosomes ascertained pre-natally (4). Patients with small derivatives of chromosome 15 have a normal phenotype, while sSMCs derived from chromosomes 13, 21 and 14 also appear to have a low risk of abnormalities (5).

Studies of the cytogenetic and phenotypic effects of sSMCs gained a fresh impetus when fluorescence *in situ* hybridization (FISH) was utilized to identify their chromosomal origin (6). At present, molecular cytogenetic methods enable precise characterization of such chromosomal abnormalities necessary for establishing their genetic risk. Herein, we report the pre-natal identification and characterization of 23 sSMCs from a series of approximately 28,000 pre-natal samples, which were assessed using various multicolor FISH techniques.

Materials and methods

Four diagnostic genetics laboratories in Greece during the period of January 2005 until October 2009 detected a total of 23 SMCs among ~28,000 diagnostic pre-natal samples. All cases were detected in amniotic fluid cultures. In cases with mosaicism, the abnormal cell line characterized by the sSMC was present in at least two independent cultures. Parental chromosome studies were performed in all cases to determine if the marker chromosome was inherited or *de novo.*

FISH analysis was performed according to a procedure previously described by Liehr *et al* (31). Centromere-specific multicolor FISH (cenM-FISH) was performed as described by Nietzel *et al* (32), acrocentric specific multicolor FISH (acrocenM-FISH) as described by Trifonov *et al* (33) and multicolor FISH with whole chromosome paint (wcp) probes was used to determine the origin of the sSMC (1,34). These results were confirmed by FISH with centromere-specific probes. Identification of euchromatic material was performed by subcentromere-specific multicolor FISH (subcenM-FISH) (3). Multicolor banding (MCB) was performed according to Chudoba *et al* and Liehr *et al* (34,35), as well as the application of locus specific probes (36). Centromeric probes and locus-specific probes were commercially available (Abbott/ Vysis, Kreatech and Q-Biogene). In addition, BAC probes derived from BAC-PAC-CHORI were applied in one case. The nomenclature of the sSMCs was according to Liehr (37).

Uniparental disomy (UPD) testing was systematically carried out using genomic DNA from parental blood lymphocytes and amniocytes. DNA was extracted using the QIAamp DNA Mini kit (Qiagen Inc., Chatsworth, CA, USA). UPD was investigated by parent-to-fetus segregation analysis using a panel of six highly polymorphic short tandem repeat (STR) markers located along the involved chromosome. UPD was considered when at least two informative markers indicated the presence of uniparental inheritance and at least five other STRs mapping to other chromosomes confirmed paternity (38). The PCR products were separated on a fluorescent capillary system (ABI PRISM 310, Foster City, CA, USA) after fluorescent PCR using one ABI-dye labeled primer.

Results

A total of 23 marker chromosomes were referred for characterization among ~28,000 diagnostic pre-natal samples. Table I summarizes the results of cytogenetic, FISH and UPD studies.

In all cases, the FISH diagnosis was available in a clinically relevant time. The mean maternal age was 36.2 years (range 27-43) and the mean gestational age at which amniocentesis was performed was 18.3 weeks (range 16-23). Eighteen markers were *de novo* and 5 markers were inherited (maternal). Of the 5 inherited markers, only one showed mosaicism (case 3, Table I) and all these cases had a normal outcome. In total, 17 markers were derived from acrocentric chromosomes (14, 15, 21 and 22) and 6 markers were non-acrocentric, derived from chromosomes 9, 16, 18, 20 and Y. Termination of pregnancy was performed in 9 cases and in 1 case an intrauterine death was reported at 21 weeks of gestation (Table I, case 7). In 7 out of the 10 cases, abnormal ultrasound findings were reported, whereas the 3 remaining cases were due to advanced maternal age (without abnormal ultrasound).

Markers derived from chromosome 15. A total of 8 markers originated from chromosome 15. Cases 1 and 2 were *de novo* mosaic bisatellited inv dup(15)(q11.1) sSMCs. The pregnancies resulted in 2 healthy male infants. Cases 3 and 4 presented a maternally inherited bisatellited marker derived from chromosome 15. The marker chromosome consisted exclusively of heterochromatic material, inv dup(15)(q11.1); 2 normal male infants were born at term. In case 5, the additional marker chromosome consisted of heterochromatic and euchromatic material and was characterized as inv dup(15)(q11.2) *de novo*. A normal female infant was born at term. The FISH is illustrated in Fig. 1A.

Cases 2, 3 and 5 were negative for the Prader-Willi syndrome (PWS)/Angelman syndrome (AS) critical region. In case 6, the marker contained the PWS/AS critical region, inv dup(15)(q14). The marker was *de novo* and the pregnancy was terminated.

In case 7, the additional marker was characterized as a derivative of chromosome 15 with two different variants of the derivative, both asymmetric. In the first variant, only one centromere-near BAC in 15q11.2 (RP11-307C10) was present, and was therefore described as dic(15)(q11.1;q11.2). In the second variant, the BAC was not present and the derivative chromosome was larger than a simple inv dup(15)(q11.1), and was described as dic(15)(q11.1;q11.1~q11.2). This *de novo* marker consisted of heterochromatic and euchromatic material and was negative for the chromosome LSI UBE3A/PML probe; intrauterine death took place at 21 weeks.

Case 8 was a *de novo* mosaic marker chromosome derivative of chromosome 15. The marker chromosome definitely contained no alphoid DNA. According to standard theories, a neocentric sSMC from the proximal part of a chromosome arm should be a ring chromosome. However, a double ring was observed, which is unusual for a neocentric ring. By CENP-antibody analysis, only one centromere was observed on the sSMC. The marker consisted of both heterochromatic and euchromatic material. A normal female infant was born at term and the cytogenetic result was confirmed in blood leukocytes.

Acrocentric markers not involving chromosome 15. Cases 14 and 15 (unrelated) were maternally inherited inv dup markers derived from chromosome 22. The marker chromosomes consisted exclusively of heterochromatic material. Two normal male infants were born at term.

Table I. List of the sSMCs detected and the outcome of the subsequent pregnancies.

Case	Age (years)	Gestation age (W) /indication	Karyotype	FISH probes/ basic clones	Identified sSMC	Pregnancy outcome
1	38	18.3/AMA	47,XY,+mar(60)/46,XY(40) de novo	cenM-FISH, subcenM-FISH mix15	inv dup(15)(q11.1)	Normal male
2	38	18.0/AMA	47,XY,+mar(85)/46,XY(15) de novo	cenM-FISH, subcenM-FISH mix15 cep 2, cep 13/21, cep 15, cep 9, SNRPN, PML	inv dup(15)(q11.1)	Normal male
3	38	17.3/AMA	47,XY,+mar(75)/46,XY(25) mat	cenM-FISH, subcenM-FISH mix15 cep 2, cep 13/21, cep 15, cep 9, SNRPN, PML	inv dup(15)(q11.1~q11.2)	Normal male
4	38	19.3/AMA	47,XY,+mar(100%) mat	cenM-FISH, subcenM-FISH mix15	inv dup(15)(q11.1)	Normal male
5	38	18.1/AMA	47,XX,+mar(58)/46,XX(2) de novo	cenM-FISH, subcenM-FISH mix15 SNRPN, PML and cep 15 (β-sat)	inv dup(15)(q11.2)	Normal female
6	33	20.2/ABN UTS Asymetric IUGR	47,XX,+mar(100%) de novo	acrocenM-FISH, subcenM-FISH mix15 LSI SNRPN, cep 15	inv dup(15)(q14)	Terminated
7	38	19.4/AMA	47,XX,+mar(100%) de novo	cep 13/21, cep14/22, cep 15, subcenM-FISH mix15 LSI UBE3A/PML	dic(15)(q11.1;q11.1~11.2)(6)/ dic(15)(q11.1;q11.2)(9)	Intrauterine death
8	35	20.1/AMA	47,XX,+mar(38)/46,XX(12) de novo	cenM-FISH, cep1/5/19, cep 1, cep 4, cep 14/22, cep 15, midi 54 M-FISH using all 24 whole chromosome painting probes, microdissection and rev. painting subcenM-FISH mix15 BAC probes RP11-307C10 in 15q11.2, RP11-138C5 in 15q12, RP11-261B23 in 15q13.2	neo r(15)(::q11.2->q13.1:: q11.2->q13.1::) or r(15) (::q13.1->q11.2::q11.2-> q13.1::) or neo min (15) (:q13.1->q11.2->q11.2->q13.1:)	Normal female
9	35	20.1/ABN UTS Bilateral club feet	47,XX,+mar (100%) de novo	cenM-FISH, subcenM-FISH mix18, subtelomere probe for 18p and cep 18	i(18)(p10)	Terminated
10	35	21.2/ABN UTS IUGR	47,XX,+mar (100%) de novo	cenM-FISH, subcenM-FISH mix18, subtelomer probe for 18p and cep 18	i(18)(p10)	Terminated
11	43	19.6/AMA	47,XX,+mar(4)/46,XX(22) de novo Cord blood 50%	cenM-FISH, subcenM-FISH for chromosome 9	i(9)(p10)	Terminated
12	38	20.0/AMA	45,X(3)/46,X,+mar(21)/ 46,XY(18) de novo	cenM-FISH, subcenM-FISH mixY, LSI SRY subtel XpYp	inv dup(Y)(q11.21)	Normal male

Table I. Continued.

Case	Age (years)	Gestation age (W) /indication	Karyotype	FISH probes/ basic clones	Identified sSMC	Pregnancy outcome
13	36	16.0/AMA	47,XY,+mar(25)/46,XY(75) *de novo*	cenM-FISH, cep 1/5/19, cep 7, cep 9, cep 13/21, cep 20, cep 18, subtel 18 wcp 20, subtel 18p, subcenM-FISH mix18, 20, MCB for 18	r(20)(::p1?2→q11.1::)(5)/ r(20)(::p1?2→q11.1::q11.1→p1?2::)(2)/ min(20)(::p1?2→q11.1::q11.1→p1?2::)(1)/	Normal male
14	36	16.3/AMA	47,XY,+mar (100%) mat	acrocenM-FISH, subcenM-FISH mix22	r(22)(::p1?2→q11.1::)(2)/min(22) (pter→q11.1)(1)/inv dup(22)(q11.1)(6)	Normal male
15	35	18.0/AMA	47,XY,+mar (100%) mat	acrocenM-FISH, subcenM-FISH mix22	inv dup(22)(q11.1)	Normal male
16	30	16.1/ABN UTS Early IUGR	47,XY,+mar (100%) t(11;22)(q23;q11.2) mat	DiGeorge/VCFS (TUPLE1) TelVysion11q	der(22)t(11;22)(q23;q11.2)	Terminated
17	27	23.4/ABN UTS Diaphragmatocele Microcephaly	47,XY,+mar (100%) t(11;22)(q23;q11.2) mat	DiGeorge/VCFS (TUPLE1) TelVysion11q	der(22)t(11;22)(q23;q11.2)	Terminated
18	31	22.4/ABN UTS IUGR, Micrognathia Cleft palate	47,XX,+mar (100%) t(11;22)(q23;q11.2) mat	DiGeorge/VCFS (TUPLE1) TelVysion11q	der(22)t(11;22)(q23;q11.2)	Terminated
19	35	16.0/AMA	47,XX,+mar (100%) *de novo*	cenM-FISH, subcenM-FISH mix14	inv dup(14)(q11.1)	Normal female
20	38	16.4/AMA	47,XY,+mar (100%) *de novo*	cenM-FISH, subcenM-FISH mix14	inv dup(14)(q11.1)	Normal male
21	39	16.0/AMA	47,XY,+mar (100%) mat	cenM-FISH, subcenM-FISH mix14	inv dup(14)(q11.1)	Normal male
22	40	17.0/ABN UTS Increased NT (6.0 mm)	47,XY,+mar (100%) *de novo*	cenM-FISH, subcenM-FISH mix16 Cep 9, cep 11, cep 16	min(16)(:p11.1→q11.2:)	Terminated
23	38	16.6/AMA	47,XX,+mar (50)/ 46,XX, (50) *de novo*	wcp for 21 and wcp for 12 subcenM-FISH mix21	i(21)(q)	Terminated

FISH, Fluorescent *in situ* hybridization; MCB, multicolor banding; AMA, advanced maternal age; ABN UTS, abnormal ultrasound; NT, nuchal translucency; VCFS, velocardiofacial syndrome.
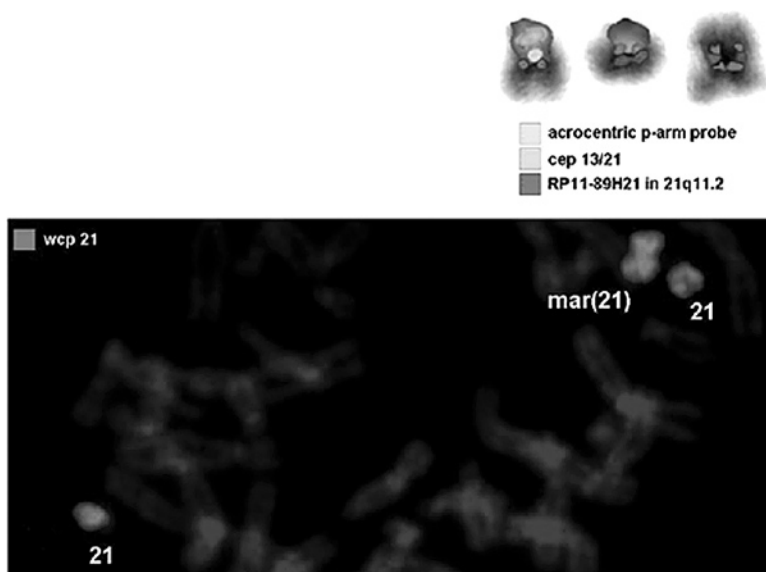
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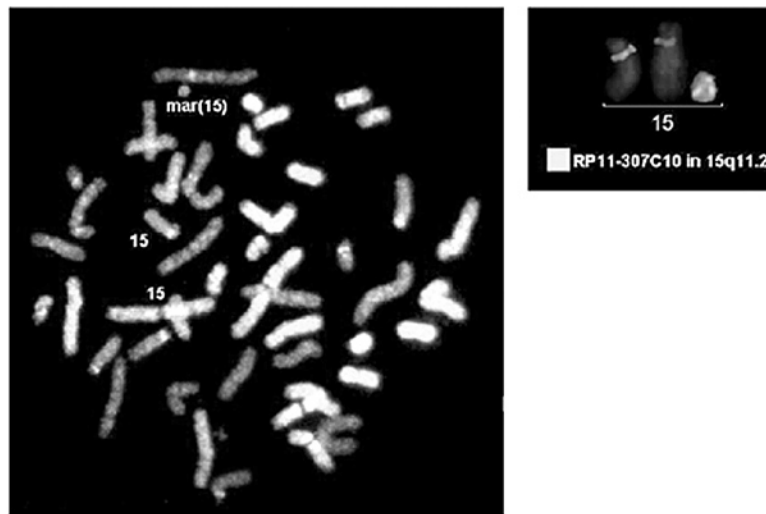
B

Figure 1. (A) M-FISH identifying the sSMC as a der(15) and FISH applying a BAC probe specific for 15q11.2 showing a ring with four signals of this specific probe. (B) Wcp probe for chromosome 21 indicating that the sSMC is an i(21)(q). SubcenM-FISH demonstrating that no p-arm material is present on the marker chromosome.

Cases 16, 17 and 18 represented derivatives of chromosome 22 [der(22)t(11;22)(q23;q11.2)]. The unrelated mothers were carriers of a balanced constitutional t(11;22) translocation and the pregnancies were terminated.

Cases 19, 20 and 21 were non-mosaic marker chromosomes originating from chromosome 14, and consisted exclusively of heterochromatic material, inv dup(14)(q11.1). Cases 19 and 20 were *de novo* and case 21 was maternally inherited. Two normal males and a normal female infant were born at term. Molecular studies showed biparental inheritance of the normal chromosome 14 homologues.

Case 23 was a *de novo* mosaic metacentric marker chromosome. The marker was positive with chromosome 21 probes. The pregnancy was terminated. The FISH is illustrated in Fig. 1B.

Non-acrocentric markers. Cases 9 and 10 were *de novo* non-mosaic metacentric marker chromosomes, positive with the chromosome 18 probes; the pregnancies were terminated.

Case 11 was a *de novo* mosaic metacentric marker chromosome. FISH with the chromosome 9 paint probe demonstrated that the marker was an isochromosome 9p; the pregnancy was terminated and the cytogenetic result was confirmed in an umbilical cord blood sample.

Case 12 was a *de novo* mosaic marker chromosome derived from chromosome Y, with 50% of the cells examined containing the Y-derived marker, 7% having monosomy X (45,X) and the remaining 43% having a normal karyotype (46,XY); a normal male infant was born at term.

Case 13 was a *de novo* mosaic ring marker chromosome derived from chromosome 20. Three distinguishable sSMCs (cryptic mosaicism), all derived from chromosome 20, were detected, including ring and minute chromosomes. This heterogeneity could not be detected by conventional G-banding or FISH techniques, but was identified by application of subcenM-FISH. The sSMC present in 25% of the cells was identified as r(20)(::p12.2~12.3->q11.1::)[5]/r(20;20)(::p12.1->q11.1::q11.1->p12.1::)[2]/min(20;20)(:p12.1->q11.1::q11.1->p12.1:)[1].

The patient had normal pre- and postnatal development and did not present any unusual phenotypic features (21).

Case 22 was a *de novo* mosaic centric minute marker chromosome derived from chromosome 16. The marker consisted exclusively of heterochromatic material and was characterized as min(16)(:p11.1->q11.2:); the pregnancy was terminated due to the measurement of increased NT (6.0 mm).

Discussion

Herein, we report 23 cases of sSMCs detected among 28,000 pre-natal samples which were characterized in detail for their euchromatic centromere-near content and a possible UPD of the relevant chromosome. This accounts for a frequency of 0.88 per 1,000 pre-natal samples, which is consistent with that reported by Ferguson-Smith and Yates (7), Hook and Cross (11), Warburton (29), Li *et al* (8), Bartsch *et al* (9), Huang *et al* (10) and Liehr and Weise (2).

It has been shown that *de novo* marker chromosomes are associated with an increased risk of mental retardation and/ or physical anomalies (1,5,11). Pre-natally ascertained cases of sSMC that have arisen *de novo* are particularly difficult to connect clinically to a phenotype. The variable euchromatin content poses a great dilemma for genetic counseling when a marker chromosome is detected during pre-natal diagnosis, as prediction of the pregnancy outcome is difficult. The correlation of a specific sSMC with a distinct clinical phenotype has been possible for some syndromes, for example the i(18p) syndrome, i(12p) Pallister-Killian syndrome (PKS), der(22) syndrome and inv dup(22) cat-eye syndrome (4). In general, the risk for an abnormal phenotype in pre-natally ascertained *de novo* cases with a sSMC is given as 7% for sSMCs from chromosomes 13, 14, 21 and 22, and 28% for the non-acrocentric chromosomes (4). Approximately 34% of the sSMC cases are correlated with known syndromes (1). PKS associated with i(12p) is present in almost 11% and i(18p) in approximately 6% of sSMC cases. A derivative chromosome 22 [der(22)t(11;22)(q23;q11.2)] represents another almost 10% of the sSMCs (1). An inv dup(22) chromosome, associated with the cat eye syndrome, is present in approximately 7% of the cases with sSMC (2). Cases with inv dup(18) are defined as the isochromosome 18p syndrome and represent a well-delineated chromosomal syndrome with detrimental phenotypic consequences (12-15).

In the present study, it was possible to associate the sSMC with a known syndrome in 7 cases; i(9p) syndrome (case 11), i(18p) syndrome (cases 9 and 10), der(22) syndrome (cases 16, 17 and 18) and i(21q) (case 23).

In case 7, the *de novo* marker chromosome was described as dic(15)(q11.1;q11.1~11.2) and consisted of heterochromatic and euchromatic material. It is not clear whether the intrauterine death occurred as a result of the sSMC (autopsy not performed).

In case 22, with the marker chromosome characterized as a centric minute chromosome 16, the mother was referred due to advanced maternal age and increased NT (6.0 mm). It is not certain whether the increased NT was caused by the sSMC (no fetal autopsy).

The dic(15)s in cases 1, 2, 3, 4 and 5 lacked the PWS/ AS chromosomal region, and UPD studies in 4 of the cases showed a biparental inheritance of the normal chromosome 15 homologues. All pregnancies resulted in healthy newborns. It is well described that sSMC(15) containing the PWS/AS chromosomal region (case 6) is associated with abnormal phenotype (6,10,16,17). Patients with sSMC(15) may also exhibit uniparental disomy (UPD15), responsible for the presence of Prader-Willi or Angelman syndromes in these patients (18-20).

In case 13, cytogenetic analysis of amniotic cells revealed two cell lines (21). The karyotype was 46,XY in 75% of the analyzed mitoses, while an additional monocentric chromosome (marker) was noted in 25% of the cells [karyotype 47,XY,+mar(25%)/46,XY(75%)]. The parental karyotypes were normal and non-paternity was also excluded. Thus, the marker chromosome probably arose *de novo*. FISH analysis using centromere-specific multicolor FISH probes showed that the sSMC was present in 25% of the cells as a cryptic mosaic (three distinguishable sSMCs, all derived from chromosome 20): r(20)(::p12.2~12.3->q11.1::) [5]/r(20;20)(::p12.1->q11.1::q11.1->p12.1::)[2]/min(20;20) (:p12.1->q11.1::q11.1->p12.1:)[1]. A normal male infant was born and has been followed up to the age of 1 year. Since the first report of an sSMC(20) by Callen *et al* (22), a total of 42 cases (including the present case) have been described, most of them detected post-natally (23). The first two patients with r(20) mosaicism detected pre-natally (24) were phenotypically and developmentally normal at the age of 20 months. The first patient had a ring chromosome containing a small amount of euchromatic material, while the second patient was a carrier of a small metacentric and most probably heterochromatic marker. A similar case was reported by Cotter *et al* (25), with a karyotype of 47,XY,+mar[3]/46,XY[17] reported to be phenotypically normal at birth. The fourth case of a prenatally detected 46,XY/47,XY,+r(20)/47,XY,+20, showed delayed psychomotor development, physical anomalies and growth retardation at the age of 16 months (26). Our patient had normal pre- and postnatal development up to the age of 1 year and did not present any of the abnormal phenotypic nor ophthalmologic features of the described cases of mosaic ring chromosome 20, nor psychomotor delay (27). However, further developmental follow-up is warranted.

In cases 16, 17 and 18, cytogenetic analysis showed a 47,XY,+der(22)t(11;22)(q23;q11.2) karyotype in all the cells analyzed, and the phenotypically normal mothers carried the t(11;22), which is the most frequently identified reciprocal translocation in humans. Carriers of the balanced constitutional t(11;22) are phenotypically normal, but at risk of having progeny with the supernumerary der(22)t(11;22) karyotype, which includes severe mental retardation (28). This represents the resulting 3:1 meiotic segregation with tertiary trisomy, i.e., der(22) as the SMC (28).

In cases 19, 20 and 21, an inv dup(14)(q11.1) was detected with biparental inheritance of the normal 14 homologues. In the literature, around 15 cytogenetically similar cases have been described, which did not show any clinical abnormality. Two additional clinically abnormal cases have been reported with inv dup(14)(q11.1), one with paternal and one with maternal UPD14 (22).

Case 12 carried a Y-derived marker in 50% of amniocytes and resulted in a normal male infant. In similar cases, the level of mosaicism is directly associated with the phenotypic

expression, which may vary from normal male genitalia or ambiguous genitalia to Turner syndrome, with an associated increased risk of infertility and/or gonadoblastoma (8).

In our study, 5/23 (21.8%) cases were inherited (maternal), in comparison to 30% reported previously (2,9,10). With regard to mosaicism, our incidence rate was 39% as compared to previous large studies indicating that 52% of sSMCs studied by cytogenetics are mosaic (Liehr *et al*, unpublished data). The majority of the mosaic cases (7/9) had a normal outcome in our series (Table I), but it is generally believed that the mosaic state does not alter the clinical outcome (Liehr *et al*, unpublished data). Termination of pregnancy was performed in our series in 10/23 cases (43.5%), which is in accordance with previous studies (29,30).

In conclusion, molecular cytogenetics combined with other molecular studies (UPD) provide valuable information on the chromosomal origin and the composition of pre-natal marker chromosomes. FISH methods are highly suited for this purpose. SMCs have been successfully characterized by wcp, cenM-FISH, MCB or combined chromosome microdissection and reverse FISH approaches. Especially in cases with normal ultrasound, the FISH techniques render genetic counseling possible when previously they were considered to be a diagnostic problem. With new technologies, such as array-comparative genomic hybridization, a full characterization of the genetic content of the markers should be attempted, and the clinical phenotype of markers in specific cases should be described/predicted. However, sSMCs that occur in a low percentage of cells may not be detectable by array-based techniques.

Acknowledgements

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