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Unexpected results in the constitution of small supernumerary marker chromosomes

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ABSTRACT

Traditional approaches for the classification of Small Supernumerary Marker Chromosomes (sSMC), mostly based on FISH techniques, are time-consuming and not always sufficient to fully understand the true complexity of this class of rearrangements. We describe four supernumerary marker chromosomes that, after array-CGH, were interpreted rather differently in respect to the early classification made by conventional cytogenetics and FISH investigations, reporting two types of complex markers which DNA content was overlooked by conventional approaches: 1. the sSMC contains non-contiguous regions of the same chromosome and, 2. the sSMC, initially interpreted as a supernumerary del(15), turns out to be a derivative 15 to which the portion of another chromosome was attached. All are likely derived from partial trisomy rescue events, bringing further demonstration that germline chromosomal imbalances are submitted to intense reshuffling during the embryogenesis, leading to unexpected complexity and changing the present ideas on the composition of supernumerary marker chromosomes.

1. Introduction

Small Supernumerary Marker Chromosomes (sSMC) are, by definition, structurally rearranged chromosomes that cannot be identified unambiguously by conventional chromosome-banding alone. Traditional approaches for their characterization such as twenty-four color FISH, centromere or subcentromere-specific multicolor FISH and microdissection followed by reverse FISH, are time-consuming and can result in ambiguous classification or misclassification of sSMC [1]. An exception is represented by the inv dup(15), the i(12p), the i(18p), and the inv dup(22) that are easily identifiable by specific cytogenetics staining (DA-DAPI) and/or FISH techniques and whose clinical outcome is well known. For the remaining ones, even when the marker is very small and with satellites at both ends, the doubt remains if it is really constituted by heterochromatin only or it contains euchromatic material with dosage-sensitive genes as well. Obviously this issue can be burning in prenatal diagnosis both for *de novo* markers and for those inherited from a normal parent. In the latter case the marker may have clinical consequences when hiding alterations in imprinted genes or when it occurs in mosaic in the parent but not in the fetus.

According to conventional cytogenetics experience, the pericentromeric region of a given chromosome constitutes most of the sSMCs, which may or may not have a ring conformation. More rare markers derive from a chromosomal portion that doesn't contain any centromere and their preservation in mitosis is granted by a neocentromere acquisition [2,3]. Other marker chromosomes are the product of 3:1 segregation of a reciprocal translocation. The most representative among them is the der(22)t(11; 22)(q23; q11) that is usually inherited by a parent carrying the reciprocal translocation [4]. In the few other similar cases the parent was always

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carrying a reciprocal translocation leading to a peculiar pachytene diagram predisposing to 3:1 segregation [5,6]. Here we describe two *de novo* sSMCs detected in fetuses with echographic abnormalities and two *de novo* sSMCs identified in children with intellectual disability. All markers have been molecularly defined through oligonucleotide genome-wide array-CGH (aCGH) that in all cases revealed unexpected results in respect to the tentative interpretation made by conventional cytogenetics. Our findings support that only genome-wide arrays are able to explain the true constitution of sSMCs indeed showing, as recently demonstrated [7,8], that at least some of them are of unpredicted complexity.

2. Material and methods

2.1. Conventional cytogenetics

Conventional cytogenetic analysis was performed in all cases according to established guidelines. For fetal samples (cases 1 and 2) a resolution of about 320–400 bands was obtained after Q-banding chromosome staining on cultures of chorionic villi and/or amniocytes. GTG-banding techniques with a 550–600 band resolution were applied to stimulated blood lymphocytes from case 3 and case 4. Conventional karyotyping was also performed on blood lymphocytes of the parents of both the fetuses and patients, so to exclude the presence of the sSMC or of a balanced rearrangement. DA-DAPI staining was applied to metaphase spreads from case 2 according to standard procedures.

2.2. Molecular karyotyping

Molecular karyotyping was performed in all cases by using oligonucleotide aCGH platforms (Human Genome CGH Microarray Kit and SurePrint G3 Human Kit, both from Agilent Technologies, Santa Clara, CA), according to the manufacturer's protocol, on 500 ng of DNA of each case and of sex-matched reference DNAs (NA10851, NA15510 Coriell Cell Repositories). DNA was extracted from cultured chorionic villi (case 1), uncultured (case 1) and cultured (case 2) amniocytes, and peripheral blood lymphocytes (cases 3 and 4). For case 1, 100 ng of DNA extracted from uncultured amniocytes and of the corresponding reference DNA were subjected to whole genome amplification by using the Genomeplex WGA2 kit (Sigma–Aldrich Co., St. Louis, MO), and then 2 μg of amplified DNA were used for the aCGH experiment. Three different platforms were used in the characterization of the sSMC cases, depending on their availability at the time of the analysis. In case 1 both 44 K and 180 K platforms were applied, the first one on DNA from chorionic villi and the second one on amplified DNA from uncultured amniotic fluid cells. Cases 2 and 3 were analyzed on a 44 K and a 105 K platform respectively. In case 4 a 180 K platform was applied. Changes in DNA copy number at a specific locus were observed as the deviation of the $\log_2$ratio value from 0 of at least three consecutive probes, by using Genomic Workbench v. 5.0.14 software (Agilent, ADM-2 algorithm with a threshold of 5). Possible mosaicism percentage was evaluated by comparing the $\log_2$ratio average value of the aberrant region to a set of experimentally obtained in-house reference values. The positions of oligomers were referred to the Human Genome March 2006 (versions NCBI 36, hg18) assembly. Copy number changes not related to the sSMC and reported in the Database of Genomic Variants (http://projects.tcag.ca/variation/) were excluded from further analysis.

2.3. FISH analysis

FISH analysis was performed on metaphase spreads from case 2 by using commercially available probes: D15Z1 (specific for chromosome 15p11.2), D15S10 and SNRPN (both on 15q11.2), 129F16/SP6 and D19S238E (chromosome 19p and 19q subtelomere probes). In case 3 FISH analysis was performed by using the 15p probe (D15Z1) and centromere and subtelomere probes specific for chromosomes 15 and 20 (D15Z4, D20Z1, D15S936, 20pTEL18 and 20qTEL14). Whole chromosome painting was also applied to this case (WCP 20). All commercial probes were from Vysis, Abbott Laboratories. Abbott Park, IL. A centromere-specific multicolor FISH (cenM-FISH) was applied to case 4 according to Nietzel et al. (2001) [9]. Further FISH experiments were performed on this case by using the following probes: RP11-746M1 (17q11.2), RP11-403E9 (17p12) and cep17 (D17Z1, Abbott Laboratories. Abbott Park, IL). All the experiments were performed according to standard procedures. Metaphase spreads were analyzed by using a Zeiss Axioplan II, Imager.M1 or Imager.Z1 fluorescence microscope equipped with appropriate filter sets. Digital images were captured and stored with Isis software V3.4.0 (Metasystems, Altlussheim, Germany).

2.4. Genotyping

Genotyping of polymorphic loci was performed by PCR amplification with primers labeled with fluorescent probes (ABI 5-Fam and Hex) followed by analysis on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Primer sequences and PCR conditions are available on request.

3. Cases description and results

3.1. Case 1

A 37-year-old woman was referred for Chorionic Villus Sampling (CVS) at 11 weeks of gestation because of abnormal biochemical testing results and increased nuchal translucency (3.45 mm). Conventional cytogenetic analysis was performed on cells from both short and long term chorionic villi culture preparations. A 47,XX,+mar karyotype was found in all examined metaphases from long term culture preparations only, while short term villi preparations (18 metaphases from 24 h to 48 h cultures) showed a normal female karyotype. Both parents had normal karyotypes. Molecular characterization of the sSMC, carried out by oligo-aCGH on cultured cells, showed the duplication of two non-contiguous portions of chromosome 18 (Fig. 1a, Table 1) so that the possible configuration of the marker is: 18pter→q12.3::q23→qter. Ecographic examination performed at 15 + 6 weeks of gestation did not show abnormalities in biometric parameters and fetal movements appeared to be normal. Amniotic fluid sampling was done at 16 + 6 weeks of gestation, to exclude placental confined mosaicism. A small aliquot of amniotic fluid was used for DNA extraction and subjected to aCGH after whole genome amplification. A 47,XX,+mar karyotype was found in all examined metaphases from cultured amniocytes. aCGH on uncultured amniocytes confirmed the result obtained on cultured villi cells. In both samples the involved regions showed $\log_2$ratio values compatible with non mosaic duplications. Microsatellite analysis was performed to confirm the array results and to define the parental origin of the sSMC. All the informative microsatellites showed maternal origin of the marker (Table 2, Supplementary Fig. 1) with all four maternally derived loci having a double peak area possibly suggesting a meiosis II error.

A detailed ultrasonographic scan was performed at 18 weeks of gestation showing growth retardation, bilateral choroid plexus cysts, kyphotic spine and dysplastic semilunar valves.

Genetic counseling was offered to the couple that opted for termination of pregnancy at 19 weeks of amenorrhea. Autopsy was performed showing a female fetus small for gestational age. The

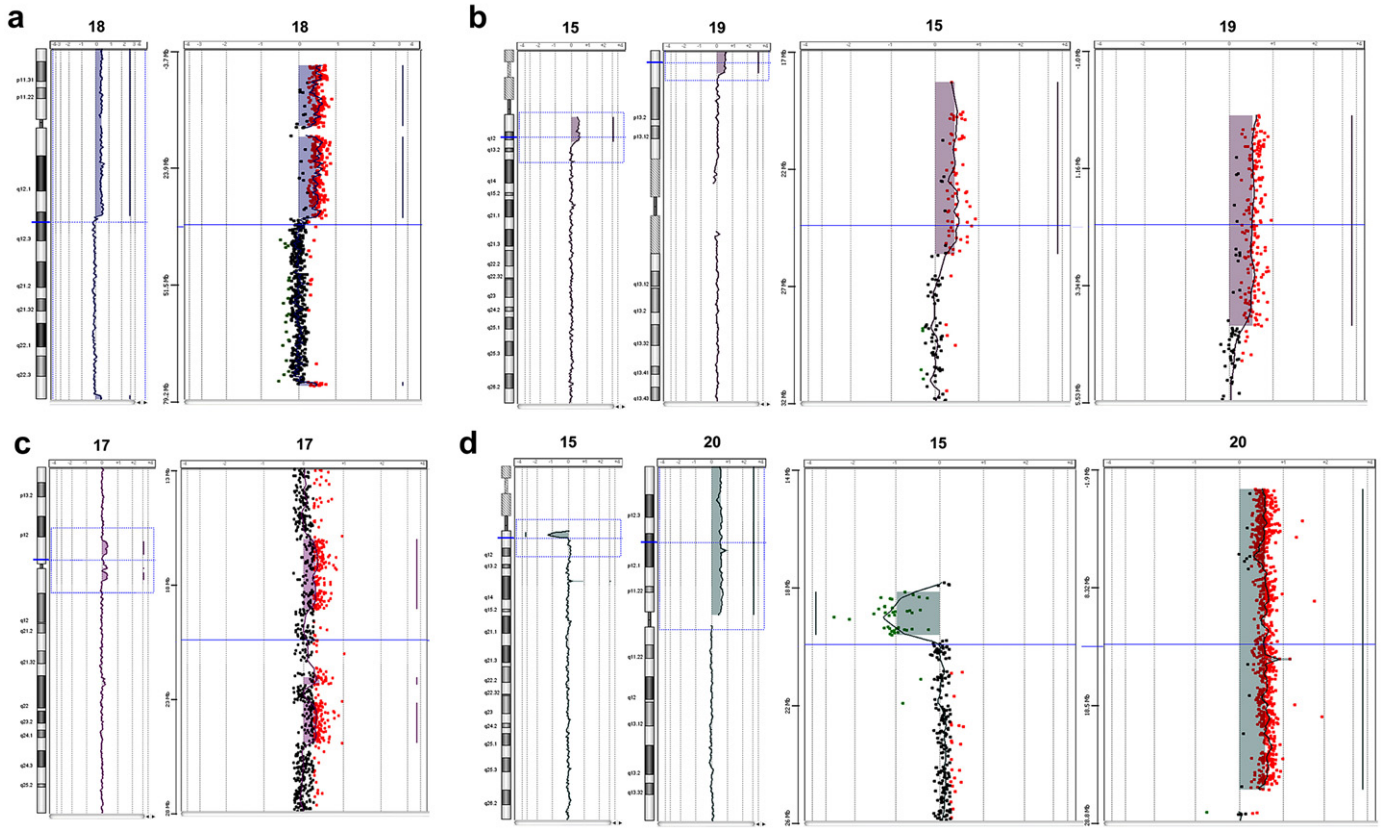

Fig. 1. Array-CGH profile of the chromosomes involved in sSMCs constitution. Chromosome view is on the left of each section. An enlargement of each involved region is on the right. a, c) The sSMC is constituted by non-contiguous portions of the same chromosome, which is chromosome 18 in case 1 (a) and chromosome 17 in case 4 (c). b, d) The supernumerary marker is a derivative 15 chromosome. For case 2 (b) the duplicated proximal chromosome 15 and distal 19p are shown. For case 3 (d) the enlargement on the right shows the polymorphic CNV observed at proximal 15q and not related to the sSMC. The short arm of chromosome 20 is entirely duplicated.

body measurements were: total weight 198 g, body crown-heel length 21 cm, crown-rump length 13 cm, head circumference 15 cm, chest circumference 14 cm, abdominal circumference 13 cm, hand length 1.5 cm and foot length 2.5 cm. The fetus presented with: oval face, low-set ears with right external ear absent and replaced by a skin fold, kyphotic spine, and marked flexion of the hand on the forearm. The examination of the internal organs showed situs solitus of thoracic and abdominal organs, dilated bowel loops and abnormal morphology and position of the left kidney. The histological study showed organ morphological findings coherent with the gestational age. The histological examination of the placenta showed immature and dysmorphic villi, and a single umbilical artery was present.

3.2. Case 2

Amniocentesis was performed at 19 weeks of gestation in a 36 year-old woman because of finding of congenital heart defect (VSD) at ultrasound scan. A *de novo* satellited and DA-DAPI positive sSMC was found in all the 30 analyzed metaphases (Fig. 2a). FISH analysis was performed with probes D15Z1, specific for the short arm of chromosome 15, and D15S10 and SNRPN, both at 15q11.2. All probes showed positive single signals on the marker excluding that it was the classical inv dup(15). Accordingly, the karyotype was interpreted as: 47,XY,+der(15)(pter→q11.2). To better characterize the marker chromosome and to define its gene content, aCGH was performed on cultured amniocytes showing a duplication of about 7 Mb of proximal 15q, spanning from 15q11.2 to 15q13.1 (Fig. 1b, Table 1). Unexpectedly, aCGH analysis also showed a 4.1 Mb duplication of distal 19p, from 19pter to 19p13.3. FISH analysis with the 19p subtelomere probe 129F16/SP6 (Vysis) confirmed the presence of this region on the marker, that was finally interpreted as der(15)t(15; 19)(pter→q13.1::p13.3→pter) (Fig. 2b).

Table 1
Genomic coordinates of the duplicated and normal regions of each sSMC chromosome of origin, as identified by oligonucleotide array-CGH (NCBI36/hg18). The asterisk denotes the first probe on the platform starting from the short arm of that chromosome. The symbol "§" denotes the last probe on the platform starting from the short arm of that chromosome.

Cytoband	Size	Start (bp)	Stop (bp)	Average $\log_2$ratio value	Status
Case 1					
18p11.32q12.3	36.1 Mb	170,229*	36,274,074	0.6	Duplicated
18q12.3q23	39 Mb	75,182,868	75,182,927	0.01	Not duplicated
18q23	921 kb	75,196,415	76,110,964§	0.6	Duplicated
Case 2					
15q11.2q13.1	7 Mb	19,109,124*	26,109,939	0.6	Duplicated
19p13.3	4.1 Mb	232,080*	4,156,366	0.6	Duplicated
Case 3					
20p13p11.1	26.1 Mb	18,580*	26,129,166	0.6	Duplicated
Case 4					
17p13.3p11.2	16.8 Mb	51,885*	16,877,328	0	Not duplicated
17p11.2	2.9 Mb	16,892,427	19,888,467	0.30	Duplicated
17p11.2p11.1	2.1 Mb	19,899,547	22,129,889	0.04	Not duplicated
17q11.1	319 kb	22,427,573	23,163,556	0.33	Duplicated
17q11.1q11.2	615 kb	23,176,239	23,841,023	−0.01	Not duplicated
17q11.2	1.8 Mb	23,848,894	25,676,268	0.31	Duplicated
17q11.2q25.3	52.9 Mb	25,683,268	78,653,545§	0	Not duplicated

Table 2
Microsatellite analysis on polymorphic STS markers for cases 1, 2 and 4. An asterisk indicates double peak area. Abbreviations: mat = maternal origin, pat = paternal origin, un = uninformative markers.

STS marker	CASE	Mother	Father	Parental origin of the duplication
	Case 1			
D18S53	173.69*/178.08	169.7/173.7	169.68/178.06	mat
D18S476	266.5*/270.37	266.5/270.35	270.27/270.27	mat
D18S54	202.03*/206.09	202.25/215.63	206.05/206.05	mat
D18S976	177.64/184.64*	181.82/184.9	177.64/184.79	mat
D18S70	101.01*/113.12	109.08/113.06	101.02/109.07	un
D18S1158	95.11/97.07*	97.13/99.04	97.06/99.02	un
	Case 2			
D15S986	179.38/183.27*	183.17/183.17	179.27/185.16	mat
D15S822	256.38/275.41/287.4	275.41/287.4	256.4/284.4	mat
D15S817	147.86/151.94*	143.7/151.8	147.9/151.9	mat
D15S1035	173.9*/230.9	173.9/227	230.9/230.9	mat
D15S1021	144.9*/148.9	133.4/144.9	143.05/148.9	mat
D20S90	198.94*/203.08	199.06/202.97	191.18/203.08	mat
D19S120	171.35*/175.52	171.35/173.38	173.46/175.5	mat
D19S209	258.39/265.95*	260.2/265.2	258.39/265.36	un
D19S424F	142.71/148.53*	148.53/154.36	142.76/148.54	un
D19S878	210.22/210.22	204.45/210.27	210.32/210.32	un
	CASE 4 – BS			
STS1	141.1/158.4	141.3/158.5	141.2/158.4	un
D17S620	142.9/147.1	143.24/147.24	138.7/142.9	un
STS3	208.5/212.6	208.6/212.6	208.6/212.9	un
D17S740	141.6/145.8	143.7/145.9	141.5/143.8	un
D17S2196	142.8/150.8/159.1	142.7/159.1	150.9/155	mat
D17S793	83.3/98.9	83.27/98.92	83.3/93.12	un

Microsatellite analysis showed the maternal origin of the additive material both for chromosome 15 and for chromosome 19 (Table 2). Both parents had a normal karyotype. Genetic counseling was offered to the couple that opted for termination of pregnancy at about 22 weeks of gestation. Autopsy showed a male fetus with normal growth parameters but dysmorphic signs: oval face, hypertelorism and long philtrum. As previously observed through ultrasound scan, a perimembranous interventricular septal defect, localized in the septal edge of the tricuspid valve, was present. Cerebral and cerebellar structures appeared normal, and no major abnormalities of the internal organs were found. Microscopic evaluation of the placenta showed immature and irregularly shaped chorionic villi with dysplasia of the stromal vessels.

3.3. Case 3 (FP)

FP was the second child of unrelated healthy parents, born premature with a birth weight of 2050 gr. She was referred to genetic evaluation because of mild mental retardation without peculiar facial dysmorphism. At the age of 9 years she presented as a shy girl attending elementary school with a support teacher. Her height was above 97th centile and she progressed normally to puberty. She had pes planus, valgism of knees and joint laxity. After cytogenetic investigations (both conventional and molecular) and a first genetic counseling, her parents decided to discontinue further investigations and were not available to perform a detailed neuropsychiatric evaluation of the proband.

Cytogenetic investigations on peripheral lymphocytes of FP showed a 47,XX,+mar karyotype with the presence of a satellited *de novo* sSMC a little smaller than a chromosome 21 (Fig. 2d). FISH analysis with the 15p probe D15Z1 suggested that the sSMC was a der15 possibly interpreted as a del(15)(pter→q13) (Fig. 2e). aCGH analysis was performed showing a small heterozygous deletion of the proximal 15q11.2 (chr15:18,668,297–20,060,061) which is a known benign variant, but surprisingly did not show any extra material on chromosome 15 even though the FISH analysis showed that the marker is originating from 15. aCGH also showed duplication of the entire short arm of chromosome 20 (Fig. 1d, Table 1). Since on the array platform repetitive genomic regions are masked, the pericentromeric regions of chromosome 15 and 20 were not deeply covered. The first probe for chromosome 15q maps at 18,362,555 bp, prompting us to assume that the sSMC only contained the short arm and the centromere of chromosome 15 whose presence was demonstrated by the previous FISH. FISH analysis with telomere and centromere-specific probes for chromosome 20, and the whole chromosome 20 painting probe set confirmed the hypothesis suggested by the aCGH results (Fig. 2f and Supplementary Fig. 2). The final interpretation of the marker was: der(15)t(15; 20)(pter→q10::p11→pter).

3.4. Case 4 (BS)

BS was the second child of unrelated healthy parents. He was born after 38 weeks of uncomplicated pregnancy. His mother underwent amniotic fluid sampling and prenatal cytogenetic analysis because of advanced maternal age (39 years). This analysis showed a 46,XY karyotype. His perinatal history was not clinically significant. At birth BS weighed 2800 g (10th centile), had a length of 50 cm (50th centile) and occipital-frontal circumference (OFC) of 34 cm (15th centile). His developmental milestones were significantly delayed as he sat unsupported at the age of 12 months, walked unaided at the age of 22 months and started uttering his first words at the age of 2.5 years, when he was referred by his pediatrician to full developmental assessment. On physical examination, at the age of 22 months, his height was 100 cm (60th centile) and his weight was 18 kg (75th centile). He presented with mild dysmorphic features such as narrow palpebral fissures, small eyes, high-arched palate, low-set ears, short hands and fingers with clinodactyly of the 5th finger. On neurological examination he was severely hypotonic with microcephaly (OFC 46 cm, <2nd centile). He was a sociable child with severe global developmental delay. He showed good ability for symbolic play, but his comprehension was limited to simple commands and his speech consisted of 2–3 simple words. His overall developmental level was equivalent to 16 months, which corresponds to a General Developmental Quotient (GDQ) of 46 according to Griffiths Scales of Mental Development. Laboratory investigation revealed a normal heart ultrasound and normal metabolic, endocrine, and biochemical screening. The visual and hearing evaluations were also normal. An MRI brain scan revealed a big arachnoid cyst close to *cysterna magna*. An early intervention program using the Portage scheme twice a week was initiated.

He was re-evaluated at the age of 3 years and 5 months. His height was 107 cm (75th centile) and his weight 20 kg (90th centile). On neurological examination he showed global hypotonia of the trunk and limbs and microcephaly (OFC 47.6 cm, <2nd centile), but without focal neurological signs. His developmental abilities had not significantly progressed. He was still severely delayed, functioning at 17 months developmental level with a GDQ less than 46. He was last time examined at the age of 5 years and 6 months when his OFC was 48 cm (<2nd centile), height 110 cm (75th centile) and weight 24 kg (90th centile). He was still a sociable boy with mild dysmorphic features and severe developmental delay. His developmental level was equivalent to a 25 months level, which corresponded to a GDQ less than 40. His speech was limited to simple two-word phrases with severe phonological immaturities. He attended a special kindergarten and received extra speech and language therapy twice a week.

Conventional cytogenetic analysis performed at the age of 2 years on blood cells lymphocytes showed an sSMC in 80 out 100 examined cells (80%). Parental karyotypes were normal. Centromere-specific multicolor FISH suggested that the mosaic sSMC was a der(17). Further FISH experiments were performed by using the

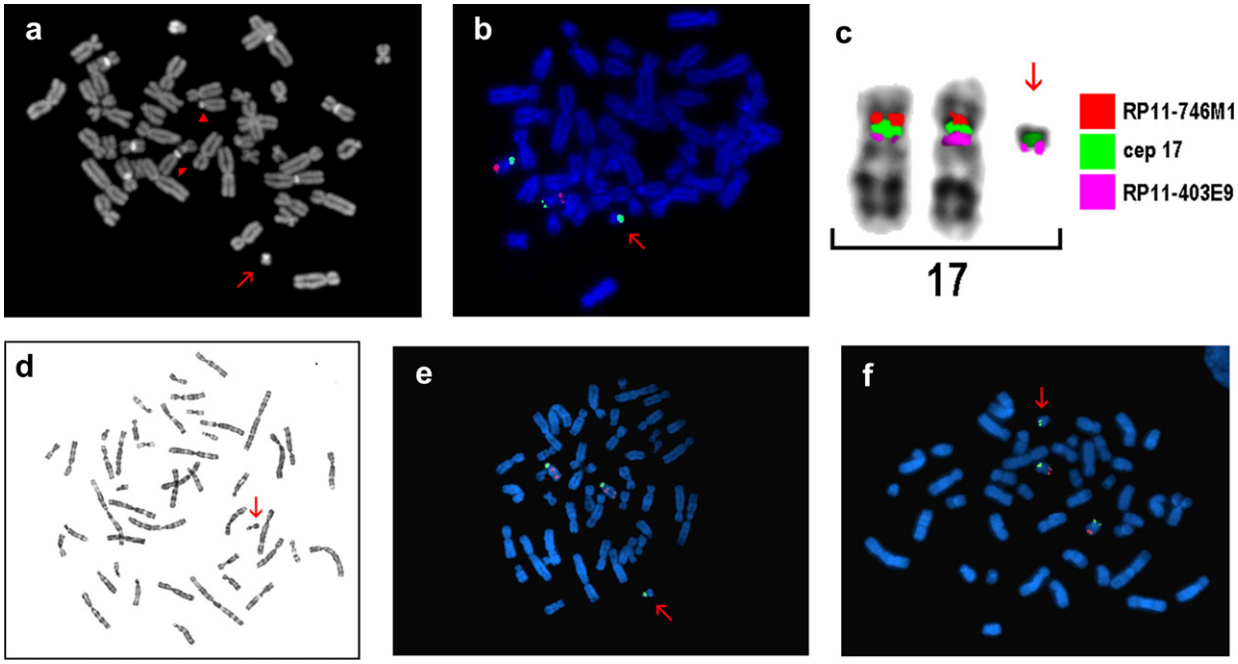

Fig. 2. Cytogenetics and FISH investigations on cases 2–4. The marker is indicated by a red arrow in each section. a–b) case 2: Da-DAPI staining on an amniotic cell metaphase from case 2 shows a strong signal on the sSMC. Arrow-heads indicate the two chromosomes 15 (a). FISH investigations with chromosome 19 subtelomeric probes 129F16/SP6 (19p, green) and D19S238E (19q, red) confirm the presence of 19p material on the sSMC (b). c) case 4: FISH characterization of the der(17) correctly identified the marker's chromosome of origin, being unable to define its complexity. The probes CEP17 (chromosome 17 centromere, green) and RP11-403E9 (17q11.2, purple), mapped on the sSMC whereas the probe RP11-746M1 (17p11.2, red) was not present. d–f) case 3: G-banded metaphase (d), FISH analysis with probes D15Z1 (15p11.2, green), D15S11(15q11-q13, red) and D15S936 (15q26.3, red) showing a green signal only on the sSMC (e), FISH with subtelomeric probes 20qTEL14 (20q, red) and 20pTEL18 (20p, green), the latter present on the sSMC.

probes RP11-746M1 (chr17:20,819,114–21,101,369), RP11-403E9 (chr17:25,520,107–25,693,302) and cep17 (D17Z1) (Fig. 2c), allowing only a partial characterization of the SMC. Molecular karyotyping was performed on DNA from whole blood of the patient, showing three non-contiguous duplications involving the pericentromeric region of chromosome 17. The three duplications, of 2.9 Mb (17p11.2), 319 kb (17q11.1), and 1.8 Mb (17q11.2) in size respectively, were separated by two single copy regions with a size of about 2.1 Mb and 615 kb respectively (see Table 1 and Fig. 1c). The average of the $\log_2$ ratio value for the duplicated regions was 0.31, suggesting a mosaicism percentage of around 70%, in agreement with what was found by routine cytogenetics. Genotyping of 6 polymorphic loci was performed on DNA from the proband and his parents. The only informative locus (D17S2196), suggested a maternal origin of the sSMC (Table 2, Supplementary Fig. 1).

4. Discussion

In this paper we describe two types of unusual markers: 1. the sSMC contains non-contiguous regions of the same chromosome (cases 1 and 4), and 2. the sSMC initially interpreted as a supernumerary del(15) was reinterpreted as a derivative 15 to which the portion of another chromosome was attached (cases 2 and 3).

4.1. Cases 1 and 4 – sSMC containing non-contiguous regions of the same chromosome

In case 1, two non-contiguous regions of chromosome 18 constitute the sSMC, that was present in all cells. Microsatellite analysis demonstrated that it had a maternal origin with double peak areas for four maternal sequence tagged sites (STSs) (Table 2, Supplementary Fig. 1). We interpreted this marker as resulting from a partial trisomy rescue of a zygote who received two maternal chromosomes 18. In fact the mother was 37-year-old at the time of conception, making likely a chromosomal non-disjunction. The finding that all the investigated STSs showed two identical alleles is not surprising since, in contrast to other trisomies, trisomy 18 typically involves maternal MII errors [10].

In case 4, three non-contiguous regions of chromosome 17 constitute the sSMC. Cytogenetic analysis on cultured amniocytes, done because of advanced maternal age (39 years), showed a normal male karyotype, whereas at two years of age both conventional cytogenetics and array-CGH showed a mosaic sSMC present in at least 70% of the proband's blood cells. The discrepancy in level of mosaicism detected by cytogenetic investigation of different tissues of the same individual is well known, possibly reflecting mechanisms of cell culturing selection. Microsatellite analysis strongly suggested a maternal meiosis I non-disjunction event, with three peaks at D17S2196. We interpreted also this case as resulting from an incomplete trisomy rescue in which a supernumerary chromosome 17 had lost about 90% of its DNA.

Chromosome shattering followed by re-conjunction of some of the broken pieces has been reported in some *de novo* constitutional complex rearrangements [11] suggesting similar mechanisms for the markers present in cases 1 and 4. Both these markers were in mosaicism, with that in case 1 not present in metaphases from two different direct chorionic villi preparations and that in case 4 apparently not present in amniocytes and present in most cells from blood. This finding indeed suggests that the original supernumerary chromosome was completely lost in some cells and partially rescued in others further supporting the massive chromosome instability occurring in the first embryogenesis [12–14].

4.2. Cases 2 and 3 – derivative 15 chromosomes

In case 2 the marker, present in all cells, is a der(15)t(15;19)(pter→q13.1::p13.3→pter). Microsatellite analysis showed that both duplicated portions of chromosomes 15 and 19 were of

maternal origin with three different peaks for D15S822 and double peak areas for the remaining six loci. Being the balanced t(15; 19) translocation not present in the parents, we assumed that the zygote had three chromosomes 15 due to maternal meiosis I non-disjunction and that one of them underwent a partial trisomy rescue with loss of most of 15q (from q13.1 to qter). The deleted 15(pter→q13) was then healed by telomere capturing [15,16] of the distal 19p13.2→pter.

In case 3 the marker, present in all cells, is constituted by a der(15)t(15; 20)(pter→q10::p11→pter) as defined by array-CGH and confirmed by FISH experiments. Unfortunately we were unable to obtain further biological material for microsatellite analysis but the mother was 39-year-old at the time of conception so it seems likely that the embryo with trisomy 15 rescued most of the supernumerary chromosome and stabilized it by telomere capture, as we assumed for case 2. We cannot exclude the opposite situation namely that the trisomic 20 embryo eliminated the long arm and the proximal short arm of the supernumerary 20 and captured the short arm of chromosome 15 containing the centromere as well.

Our data strongly suggest that all the sSMCs we describe derived from incomplete trisomy rescue events. The maternal origin in at least three of them and the maternal age increased at the time of conception in all of them indeed make likely a non-disjunction event at maternal meiosis. A significant maternal age effect in association with *de novo* SMCs has been reported since 1987 by Hook and Cross in a study population of 75,000 prenatal cytogenetic diagnoses [17]. In our four cases we have to assume that trisomy rescue, a life-saving mechanism, occurred either after fragmentation of one supernumerary chromosome and reunion of some of the pieces, one of which contained the centromere, or by partial deletion of the supernumerary chromosome followed by telomere capture to heal the broken end of the deleted chromosome. Chromotripsis, an event during which a shattered chromosome is randomly re-assembled, has recently been proposed to explain complex rearrangements with copy number gains or losses both in cancer [18] and in germline cells [11] and cannot be excluded as a mechanism occurring in early embryogenesis.

The concept that a proportion of *de novo* sSMCs is the result of a partial trisomy rescue is not new [19] and it has been estimated that *de novo* sSMCs were present in 4% [20] to 20% [21] of UPD cases. Recently, a high incidence of chromosome instability (CIN) was reported in human cleavage stage embryos [12–14]. Our findings confirm that germline chromosomal imbalances are submitted to intense reshuffling leading to an unexpected complexity. We predict that the extensive application of genome-wide array technology will reveal more surprises possibly changing present ideas on the composition of supernumerary marker chromosomes.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmg.2012.01.010.

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