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ARTICLE

Recurrent 70.8 Mb 4q22.2q32.3 duplication due to ovarian germinal mosaicism

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A mosaicism is defined by the presence of two or more populations of cells with different genotypes in one individual. Chromosomal germinal mosaicism occurs in germ cells before the onset of meiosis. Previously, few studies have described germinal mosaicism. In this study, we report on two siblings who carried identical pure and direct interstitial 4q22.2q32.3 duplication. Procedure investigations included complete clinical description, conventional cytogenetic analysis, fluorescence *in situ* hybridization (FISH), comparative genomic hybridization (CGH) array experiments and microsatellite study searching for parental origin of the duplication. Microarray CGH and further FISH experiments with BAC clones showed the same 70.8 Mb direct duplication, dup(4)(q22.2q32.3). Molecular studies of the 4q duplication were consistent with maternal origin associated with mitotic or meiotic rearrangements. This structural chromosomal aberration was associated in both cases with increased nuchal translucency, growth retardation and dysmorphy. Cardiopathy and lung malformations were only evident in the first case. These clinical manifestations are similar to those previously reported in previous studies involving pure 4q trisomy of the same region, except for thumb and renal abnormalities that were not obvious in the presented cases. The amplified region included genes involved in neurological development (*NEUROG2, MAB21L2, PCDH10/18* and *GRIA2*). The recurrent 4q duplication in these siblings is consistent with a maternal ovarian germinal mosaicism. This is the first description of germinal mosaicism for a large chromosomal duplication and highlights that genetic counselling for apparently *de novo* chromosome aberration should be undertaken with care.

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INTRODUCTION

A mosaicism is defined by the presence of two or more cell lines with different genotypes in one individual, who has developed from a single fertilized egg.[1] Different types of mosaicism exist, such as germinal mosaicism (restricted to germ cells) or tissue mosaicism. The most common form of mosaicism detected through prenatal diagnosis involves trisomies. Chromosomal germinal mosaicism is rare and occurs in early germ cells (oogonia or spermatogonia) before the onset of meiosis.[1] These mitotic errors can be nondisjunctions (abnormality of chromosome number) or the production of structural rearrangements (deletion, duplication, inversion, insertion or translocation). Germinal mosaicism becomes evident when two siblings are born with the same *de novo* chromosomal abnormality. Germinal mosaicism is independent of maternal age.[2] Few studies in literature have described germinal mosaicism corresponding essentially to aneuploidies and structural chromosomal aberrations including mostly isochromosomes and deletions[3–19] (for review, see Röthlisberger and Kotzot[20]).

In this study, we report two siblings carrying the same pure and direct 4q22.2q32.3 duplication characterized using microarray comparative genomic hybridization (CGH) and due to ovarian germinal mosaicism.

MATERIALS AND METHODS

Clinical report

The first pregnancy of a 24-year-old woman was marked by increased nuchal translucency (4.5 mm) on first-trimester ultrasound examination. Parents were nonconsanguineous and both familial histories were unremarkable. Chorionic villi were sampled for chromosomal analysis that showed a large long arm on chromosome 4. This chromosomal abnormality was confirmed on amniotic fluid analysis at 16 weeks. After genetic counselling and according to the French law, the pregnancy was terminated at 20 weeks of gestation. The female foetus was hypotrophic. Weight, height, head circumference and biparietal diameter measurements were 221.6 g (<5th percentile), 15 cm (5th percentile), 25 cm (25th percentile) and 14 cm (<25th percentile), respectively. Physical examination showed cranio-facial dysmorphism with elongated skull, large nose, long philtrum, large mouth with self-effacing cupidon arc, badly hemmed ears and neck oedema (Supplementary Figure 1). Internal examination revealed pulmonary lobulation defect with two lobes in the right lung and a single one in the left lung. Interauricular communication was also noted. The placenta was

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hypotrophic (86.8 g *versus* approximately 160 g for normal controls at the same term) but with normal aspect.

During the second pregnancy, increased nuchal translucency was again detected (5 mm) on first-trimester ultrasound examination. Chorionic villi were sampled and chromosomal analysis showed an abnormal long arm on chromosome 4 similar to that observed in the first foetus. Regular foetus ultrasound examinations showed normal amniotic fluid volume and no malformations were detected in the foetus whose growth was normal. After several discussions, both parents decided to continue the pregnancy. At 38 weeks of gestation, a boy was vaginally delivered. His weight was 3040 g (<25th percentile). Physical examination showed general hypotonia and facial dysmorphism with narrow and horizontal palpebral fissures, prominent nose and moderate microretrognatia. At 6 weeks of age, his weight, height and head circumference were 4400 g (<25th percentile), 55.5 cm (<50th percentile) and 37.5 cm (<25th percentile), respectively. The smile response was acquired, axial tonus was correct and no other abnormality was specified. Facial dysmorphic features were stable. At the age of 6 months, the boy had no specific medical treatment. His weight, height and head circumference were 7070 g (−1 SD), 64 cm (−1 SD) and 42 cm (−1 SD), respectively. He was able to catch close objects but not distant ones. He was able to burst into laughter. The seating position was not completely acquired and axial hypotonia was still present. At the age of 1 year, his weight, height and head circumference were 8900 g (−1 SD), 70 cm (−1 SD) and 46 cm (−1 SD), respectively. Axial hypotonia was still marked, resulting in mild kyphosis when seating and in the absence of any possibility to stand up. Psychomotricity support was begun. At the age of 32 months, his height, weight and head circumference were 80 cm (−3.5 SD), 10,500 kg (−2.5 SD) and 49 cm (−1 SD), respectively. The child was unable to walk alone and still needed some help. He was able to walk on all fours limbs and to stand up against a wall or a table. His language was very poor, limited to two or three disyllabic words, whereas hearing explorations proved normal. Facial dysmorphism was unchanged (Supplementary Figure 2). Educational support was continued.

Conventional cytogenetic analysis

Chromosome analyses were performed from uncultured and cultured trophoblast cells, amniotic cells and peripheral lymphocytes using standard procedures (RHG banding, CBG banding and a high-resolution banding technique obtained after cell culture synchronization and BrdU incorporation).

DNA extraction

Genomic DNA was isolated from peripheral blood (parent's propositus), placenta (case 1) and cultured trophoblast cells (case 2) using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Courtaboeuf, France). Extracted DNA concentrations were estimated using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Extracted DNAs were used for array CGH (aCGH) and microsatellite analysis.

Array comparative genomic hybridization (aCGH)

The genomic imbalances of placenta and trophoblast cells were analysed by aCGH using 105K oligonucleotide arrays (Hu-105A, Agilent Technologies, Massy, France). All array hybridizations were performed according to the manufacturer's recommended protocols. In brief, 3 μg of genomic DNA was digested with Alu*I* (5 units) and Rsa*I* (5 units) for 2 h at 37 °C and fluorescently labelled with the Agilent Genomic DNA labelling kit PLUS (Agilent Technologies). A male or female human genomic DNA (Promega, Charbonnière, France) was used as reference. Experiments were conducted in dye-swap. Cy5-dUTP patient DNA and its gender-matched reference labelled with Cy3-dUTP were denatured and preannealed with Cot-1 DNA and Agilent blocking reagent before hybridization for 40 h at 20 r.p.m. in a 65 °C rotating hybridization oven (Agilent Technologies). After washing, the slides were scanned on an Agilent Microarray Scanner. Captured images were processed with Feature Extraction 9.1 (Agilent Technologies) software and data analysis was performed with CGH Analytics 3.5 (Agilent Technologies). Copy number variations (CNVs) were considered significant if they were defined by three or more oligonucleotides spanning at least 50 Kb and contained at least one gene and were not identified in the Database of Genomic Variants.

Fluorescence *in situ* hybridization (FISH)

FISH analyses were performed on trophoblast cells and metaphase spreads from both parental and propositus lymphocytes. The whole-chromosome painting (WCP) probe specific for chromosome 4 was used according to the manufacturer's recommendations (Amplitech, Compiègne, France).

BAC clones specific for the 4q chromosomal region (RP11-397E7 located at 4q21.3, RP11-79M20 located at 4q22.1, RP11-451M10 located at 4q22.1, RP11-16I17 located at 4q22.3, RP11-81J9 located at 4q25, RP11-501E13 located at 4q25, RP11-77P11 located at 4q28.1, RP11-481K16 located at 4q31.21, RP11-301H24 located at 4q31.21, RP11-655B23 located at 4q31.23, RP11-177L7 located at 4q32.3 and RP11-18D7 located at 4q35) were used. BAC DNAs were labelled by nick translation using a FITC-dUTP nucleotide or Rhodamine-dUTP nucleotide (Roche Diagnostics, Rungis, France).

Microsatellite analysis

Thirteen polymorphic markers from chromosome 4 (see location and characteristics in Table 1) were coamplified by multiplex polymerase chain reaction (PCR). Primer sequences were designed according to the GeneDB locus description from The Wellcome Trust Sanger Institute. Multiplex PCR was carried out following standard protocols using 50 ng of DNA in 50 μl reaction volume and fluorescence dye-labelled primers (10 pmol each primer, 10 mM Tris-HCl (pH 9), 50 mM KCl, 2.25 mM $MgCl_2$, 0.2 mM each dNTP and 2 U Taq Polymerase (Taq Core Kit; MP Biomedicals, Illkirch, France)). PCR conditions were denaturation at 95 °C for 5 min, followed by 20 cycles of 95 °C for 40 s, 58 °C for 40 s, 72 °C for 40 s and a final 72 °C extension step for 7 min (Veriti Thermal Cycler; Applied Biosystems, Courtaboeuf, France). PCR products were

Table 1 Oligonucleotide primer characteristics for microsatellite analysis

Marker name	*Chromosome position*	*GenBank Accession number*	*Dyes*	*Forward primer (5′–3′)*	*Reverse primer (5′–3′)*	*Amplicon size (pb)*
D4S1573	4q26	Z23890	NED	ACATGGAGAATCTTTTAGTAGCA	CTTTTGAGATACCCCTATCAGT	101–113
D4S191	4q26	L15744 M84926	6FAM	AATAGGGAGCAATAAGGTGT	TTTTTATTATGTTTGCTGCTC	86–90
D4S3024	4q26	Z51607	VIC	CTGGAAGCCAGGTAGGA	AACACTTAGAACTTGCAGCC	79–99
D4S427	4q27	Z17128	VIC	GGACCTCCTTGCTTCG	CCCCTTAGGTTGCTTGT	142–166
D4S430	4q27	Z17169	6FAM	GGATGTGAGGAGTTCTGAATTTTG	ACTTTTCTGAGGACCCAGTCTTG	162
D4S194	4q28.1	M84929	VIC	CTAAGGGATGACTATATCCT	GATCTGATTACATGTCCGT	97–109
D4S2959	4q28	Z51165	VIC	AGCTTCCATGGTCATTAGAGT	TAGGGTCCTCCAAAGAACAGA	124–156
D4S422	4q28	Z17010	6FAM	GGCAAGACTCCGTCTCAA	TGAAGTAAAATTTGGGAGATTGT	75–97
D4S1579	4q28	Z23955	6FAM	CTTCCTGACTCCCACCTGGTTT	TATGCAGTCACTGGAGCATCCG	151
D4S2939	4q31.2	Z52783	PET	TTTCCACCTGGCCTTAT	CTCTTGAAGCCCTGAAGTTT	137–163
D4S3334	4q31.21	G10535	NED	GGCCAACAGAGCAGGATC	GCCAAGAGAGTGAGACTCCA	84–105
D4S1565	4q31.21	Z23855	NED	CCGTAACACAAAAAACAGATTTCAAG	GGCTGGATAACTCTGAAGAAAGG	158
D4S192	4q31.21	M84927	PET	GATCCTCAAGTGGAGTTTG	TTCAAGCACTGAAAGGGATG	85–95

then separated onto an ABI Prism 3130 analyser (Applied Biosystems), with the GeneScan 500LIZ as size standard. Data were analysed using GeneMapper 4.0 software (Applied Biosystems).

RESULTS

Conventional cytogenetic analysis

In case 1, a direct analysis of trophoblast cells showed an abnormal female 46,XX,add(4) karyotype with an additional chromosomal region located on 4q. In case 2, cytogenetic analyses on trophoblast cells revealed the same abnormal chromosome 4 with an additional region at the long arm (46,XY,add(4)) (Figure 1a). In both cases, this was interpreted as representing either a duplication of the long arm 4q or another rearrangement such as a translocation or an insertion. Analyses of parents' peripheral blood lymphocytes showed normal standard karyotypes.

DNA microarray assay

CGH analysis showed in both cases a gain of the 4q22.2q32.3 region (case 1 in Figure 1b and case 2 in Figure 1c). For both cases, the analysis revealed a proximal break point located on 4q22.2 (position 94 128 982) and a distal break point on 4q32.3 (position 164 941 617). These genomic positions were determined using version 18 of the human genome built (http://genome.ucsc.edu/). Thus, a 70.8 Mb interstitial region was amplified. Analyses revealed other variations (gain or loss) on chromosome 4 and on other chromosomes. These changes corresponded to CNVs previously reported in the database of genomic variants (http://projects.tcag.ca/variation/).

FISH analysis

The WCP probe specific for chromosome 4 showed complete hybridization on normal chromosome 4 and on the abnormal chromosome 4 (Supplementary Figure 3A). No hybridization signal was detected on any other chromosome. This finding excluded the possibility of an insertion or a translocation. FISH experiments could not be realized in case 1 because only uncultured placenta was available.

In case 2, BACs RP11-16I17 (4q22.3), RP11-81J9 (4q25), RP11-77P11 (4q28.1), RP11-481K16 (4q31.21) and RP11-655B23 (4q31.23) gave one signal on normal chromosome 4 and two signals on duplicated chromosome 4. No hybridization signal of these BAC probes was detected on any other chromosome. Moreover BACs RP11-451M10 (4q22.1), RP11-177L7 (4q32.3) and RP11-18D7 (4q35) showed one signal on both normal chromosome 4 and the duplicated one. Thus, we confirmed that the proximal break point was located between BACs RP11-451M10 (4q22.1) and RP11-16I17 (4q22.3) and the distal one between BACs RP11-655B23 (4q31.23) and RP11-177L7 (4q32.3). These results were in accordance with the propositus CGH array profile.

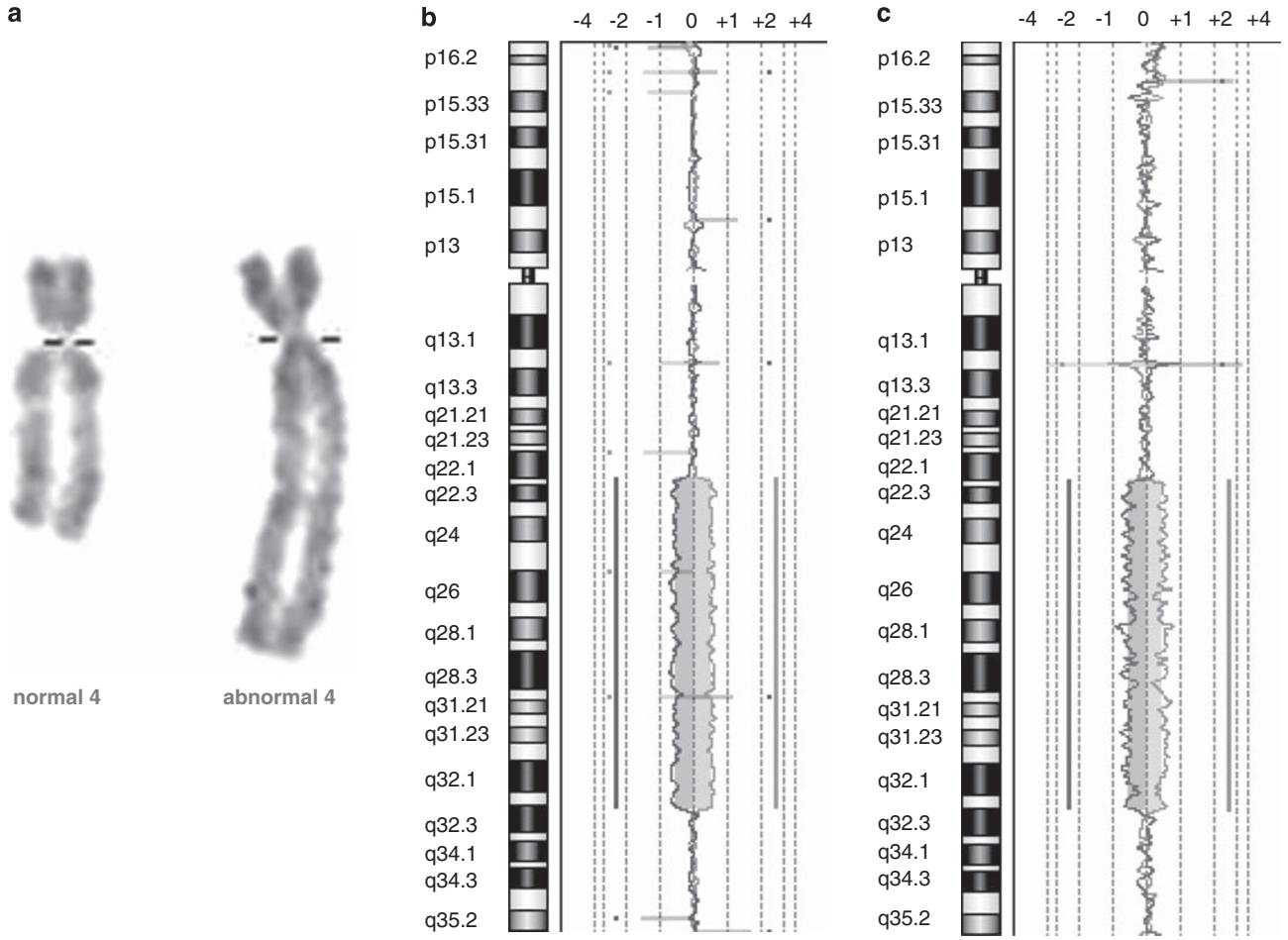

Figure 1 R-banding partial karyotype of chromosome 4 from trophoblast cells showing additional material on the long arm of one chromosome 4 (right) (**a**). Chromosome 4 DNA dye-swap profile from array-based CGH analysis showing gain (duplication) for oligonucleotides located in the 4q22.2q32.3 region for case 1 (**b**) and case 2 (**c**).

To clearly define the nature of the chromosomal rearrangement, double colour hybridization using BAC probes RP11-501E13 (4q25) and RP11-301H24 (4q31.21) was realized. The position of hybridization signals on the duplicated chromosome showed that the rearrangement was a direct duplication (Supplementary Figure 3B). Schematic representation of the position of BAC probes is summarized in Supplementary Figure 3C.

To exclude low mosaicism for the chromosome 4 duplication, FISH analyses using BACs RP11-301H24 (4q31.21) were performed in parent cells. Two signals were observed in 200 interphase nuclei and 100 metaphases for each parent, thus excluding duplication 4q mosaicism in lymphocytes.

Microsatellite analysis

Microsatellite marker analysis was informative for parental origin determination and also in specifying the mechanism of the duplication rearrangement (Table 2). Indeed, D4S427, D4S422, D4S1579 and D4S3334 marker profiles were consistent with maternal origin for both cases (Table 2). As a representative result, D4S3334 analysis is shown in Figure 2a. Moreover, D4S1573, D4S191, D4S3024, D4S427, D4S194 and D4S1579 marker analyses indicated that both duplicated maternal alleles were different for case 1 and were identical for case 2 (Table 2). These results pointed out an interchromosomal rearrangement for case 1 and an intrachromosomal one for case 2. As a representative result, D4S194 analysis is shown in Figure 2b. A possible segregation of these informative microsatellite markers illustrating this recombination is represented in Figure 3.

In summary, these conventional and molecular cytogenetic experiments allowed a precise characterization of the chromosomal formula of both siblings. We concluded that both cases carried a pure and direct dup(4)(q22.2q32.3) of maternal origin. As the maternal karyotype was normal, these results suggested an ovarian germinal mosaicism.

Table 2 Chromosome 4 microsatellite analyses

Marker	*Band*	*Father*		*Mother*		*Case 1*			*Case 2*			*Mitotic or meiotic recombination*
D4S1573	4q26	1	3	1	2	1	2	3	2	2	3	+
D4S191	4q26	1	2	2	3	2	2	3	1	2	2	+
D4S3024	4q26	1	3	1	2	1	1	2	1	1	3	+
D4S427	4q27	2	3	1	2	1	2	2	1	1	3	+
D4S430	4q27	1	2	1	1	1	1	2	1	1	1	NI
D4S194	4q28.1	1	3	1	2	1	2	3	1	1	1	+
D4S2959	4q28	1	1	1	1	1	1	1	1	1	1	NI
D4S422	4q28	1	1	2	2	1	2	2	1	2	2	NI
D4S1579	4q28	1	3	1	2	1	2	3	1	2	2	+
D4S2939	4q31.2	1	2	2	2	1	2	2	2	2	2	NI
D4S3334	4q31.21	2	2	1	1	1	1	2	1	1	2	NI
D4S1565	4q31.21	1	2	1	1	1	1	2	1	1	1	NI
D4S192	4q31.21	1	1	1	1	1	1	1	1	1	1	NI

1, 2, 3, arbitrary units for allele sizes; NI, not informative.

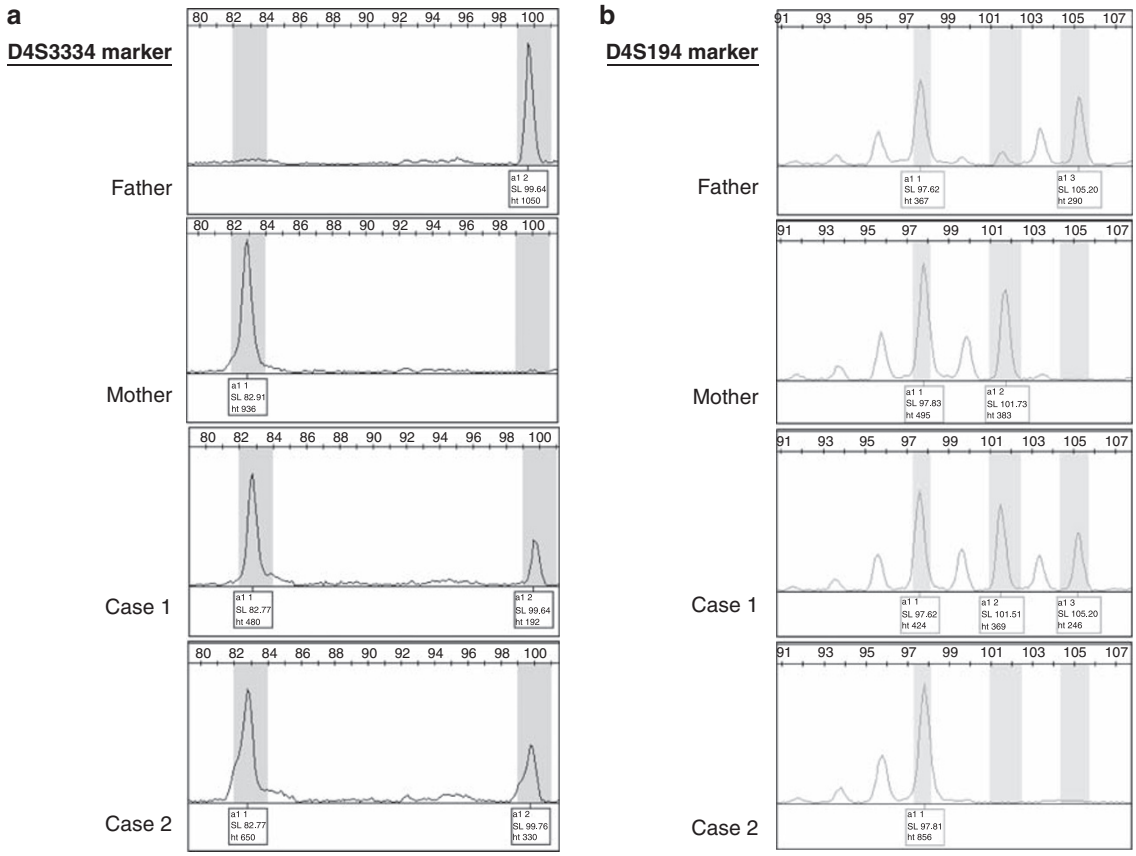

Figure 2 (**a**) D4S3334 microsatellite marker profile showing maternal origin of duplicated alleles for both cases. (**b**) D4S194 microsatellite marker profile showing an interchromosomal rearrangement for case 1 and an intrachromosomal rearrangement for case 2.

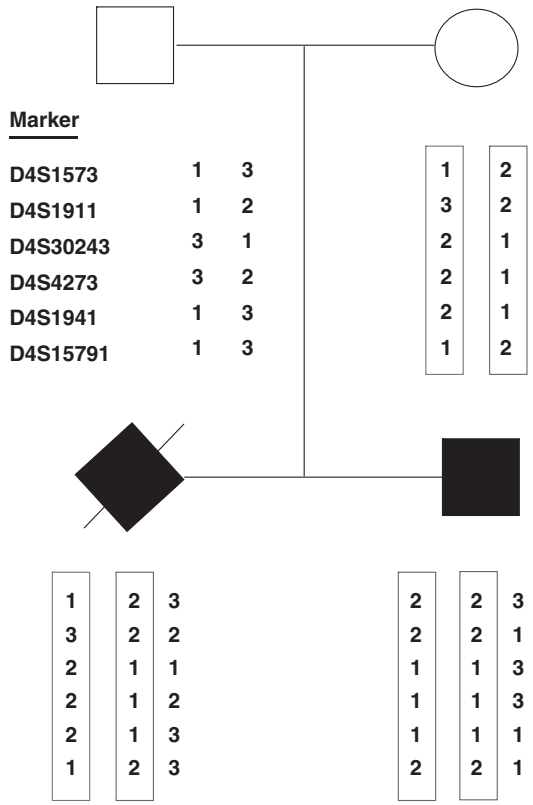

Figure 3 A possible pedigree (blood cells) of the four members of the family with haplotype analysis in the 4q26q28 region (informative markers) showing the allelic recombination. Maternal alleles are circled.

DISCUSSION

We report on two siblings (a female foetus and a 3-year-old male infant) carrying the same structural abnormality with normal parental karyotypes. In both cases, prenatal ultrasound had detected increased nuchal translucency. Both presented facial dysmorphic elements such as long philtrum, large mouth with self-effacing cupidon arc, low-set and badly hemmed ears, horizontal palpebral fissures, prominent nose, moderate microretrognatia and microcephaly. They also showed prenatal and/or postnatal growth retardation. These phenotypes were associated with a *de novo* direct dup(4)(q22.2q32.3). In both cases, CGH array indicated a 70.8 Mb duplication size and microsatellite analysis showed that the duplication had maternal origin. Microsatellite analysis also specified that the duplication resulted from an interchromosomal rearrangement in the first case and from an intrachromosomal one in the second case. Thus, these results suggest an ovarian 46,XX/46,XX,dup(4)(q22.2q32.3) mosaicism.

Germinal mosaicism is independent of maternal age,[2] and becomes evident when two siblings are born with the same *de novo* chromosomal abnormality. Numerous studies described germinal mosaicism involving molecular defects such as gene deletion, gene mutation or intragenic intron rearrangements (for review, see Zlotogora[1]). Cases of germinal mosaicism involving structural and numeric chromosomal rearrangements are rare but described (for review, see Röthlisberger and Kotzot[20]). Chromosomal germinal mosaicism is because of mitotic errors before the onset of meiosis. These errors could be nondisjunctions (abnormality of chromosome number) or the production of structural rearrangements (deletion, duplication, inversion, insertion or translocation). Few studies have highlighted parental germinal mosaicism with an abnormal number of chromosomes such as trisomy 18 and 21[3–7] or monosomy X.[3,8] Until now, only 11 studies have described germinal mosaicism involving structural chromosomal aberration.[9–19] Indeed, five studies reported structural abnormality with a maternal mosaicism origin.[9,10,11,18,19] Engel *et al*[9] reported a familial pseudodicentric chromosome (5;21) occurring on maternal germline mosaicism as revealed by microsatellite marker analysis. Another study described sibling cases with a subtelomeric 5.8 Mb deletion on chromosome 15 as a result of maternal germinal mosaicism[10] using microarray CGH and polymorphic marker analysis. A recurrent case of chromosome 18 inversion [46,XY,+18,der(18;inv(18))(q10;q10)] was also described as a result of a maternal germinal mosaic.[11] Finally, two recurrent deletions [del(16)(q11.3q12.2) and del(22)(q13.3qtel)] were reported.[18,19] With regard to male mosaics, another study reported sibling propositus with chromosome 14 structural abnormalities as a consequence of father testicular mosaicism,[12] and two more cases of recurrent paternal deletions on chromosomes 11 and 13 were described.[16,17] Our report is the first to describe a large duplication rearrangement due to germinal mosaicism. Chromosomal regions around break points did not contain segmental duplications (UCSC Genome Browser database search) that could favour the duplication mechanism. In this study, six informative microsatellite analyses indicated the occurrence of an allelic crossover during maternal gamete production.

Two hypotheses can be established to explain case 1 interchromosomal recombination and case 2 intrachromosomal recombination (Figure 4). First, allelic crossover could have occurred in the prophase of the first meiotic division (Figure 4a). Indeed, theorical meiotic recombination frequency in the duplicated region having a size of 70.8 Mb is approximately 70%.[21] Second, allelic crossover could have occurred during oogonia mitotic division (Figure 4b). However, the first hypothesis is more plausible. In both situations, four gamete types could be produced in ovaries to explain our observations. These allelic recombinations in the duplicated 4q region between siblings could explain the phenotype differences observed between both siblings by affecting, for example, gene regulation.

To our knowledge, 17 studies have described the pure 4q duplication region covering the 4q22q32 region[22–38] (total reports included the 4q21q35.2 chromosomal region analysed by conventional cytogenetic and FISH experiments, see Supplementary Table 1). No prenatal case has ever been reported.

Comparing clinical features of the 22 patients including ours (Supplementary Table 1) showed the occurrence of growth or psychomotor/mental retardation (15 out of 22 reported cases), microcephaly (11 of 22), dysmorphic elements including epicanthus folds (15 of 22), prominent nasal bridge (16 of 22) and low-set ears (14 of 22). Short philtrum (9 of 22) and micrognathia (8 of 22) are relatively common. It has been suggested that thumb and renal abnormalities could be associated with the 4q22q23 and/or the 4q25q31.3 region.[30,31] Contrary to the reports of these researchers, we did not observe thumb or renal abnormalities in our patients having a 4q22q32 duplication. To our knowledge, the present study is the first report of a pure and recurrent 4q duplication investigated with CGH microarray technology and microsatellite analysis. CGH on chromosomes has been used in the study by Elghezal *et al.*[32] CGH array analysis allowed a more precise description of distal and proximal break points because of a better resolution of 15 kb (average probe spacing). Gene analysis using the UCSC Genome Browser database (http://genome.ucsc.edu/) of CGH microarray results listed more than 110 duplicated genes. Thus, it was difficult to correlate observed phenotypes with implicated genes. Nevertheless, several

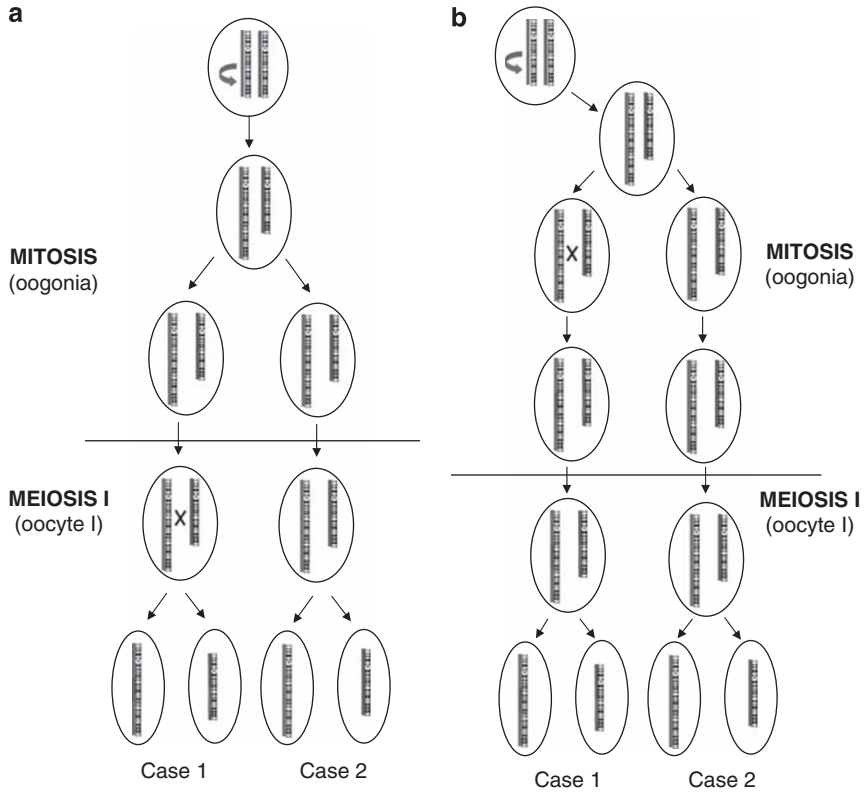

Figure 4 Schematic representation of the two hypotheses explaining chromosome 4 rearrangement during ovarian gamete production. Crossover involving the duplicated segment could result in a meiotic process (**a**) or in a mitotic process (**b**). In both situations, four identical gametes could be produced.

duplicated genes expressed in neurogenesis, such as neurogenin 2 (*NEUROG2*, 4q25),[39] mab-21-like 2 (*MAB21L2*, 4q31),[40] protocadherin 10/18 (*PCDH10*, 4q28.3 and *PCDH18*, 4q31) and glutamate receptor ionotropic AMPA2 (*GRIA2*, 4q32q33),[41] could be implicated in the phenotype. In particular, an overexpression of these genes could explain the general hypotonia and mental delay observed in the second patient.

In conclusion, this report highlighted that genetic counselling for apparently *de novo* chromosome aberration should be undertaken with care with regard to germinal mosaicism. The actual risk for *de novo* structural aberration taking into account the abortion risk of prenatal diagnostic is estimated at approximately 0.5–1%.[20]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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