ELECTRONIC LETTER

Mosaicism del(8p)/inv dup(8p) in a dysmorphic female infant: a mosaic formed by a meiotic error at the 8p *OR* gene and an independent terminal deletion event

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Various chromosomal rearrangements are associated with the distal 8p region. Among them are the inv dup(8p),[1] del(8p22),[2–5] and del(8)(pter).[6] The cardinal phenotypic features of the inv dup(8p) are brain malformations, severe mental retardation with specific involvement of speech, and minor facial dysmorphisms.[7] Deletions (8p22) are associated with congenital heart malformations, thought to be caused by haploinsufficiency of GATA4.[2] Other features are microcephaly, intrauterine growth retardation, mental retardation, and a characteristic hyperactive impulsive behaviour. The patients with del(8p) present with variable severe malformations dependent on the size of the deletion. The largest terminal 8p deletion which has been detected in a liveborn is del(8)(pter→21.1).[8]

Molecular analysis of both 8p duplications and 8p interstitial deletions showed that all cases shared similar chromosomal break points. This finding led to the hypothesis that these rearrangements were caused by ectopic recombination at misaligned duplicons. Recently, Giglio *et al*[4] showed the presence of olfactory gene clusters (*OR* clusters) at the sites where the interstitial 8p deletions occur. Whereas these interstitial deletions could thus be explained by misalignments of the *OR* clusters during meiosis, they also presented an elegant explanation for the origin of the 8p duplications. An inversion polymorphism between these *OR* clusters, present in 20% of the population, abrogates correct pairing between the *OR* clusters which causes susceptibility for an intrachromosomal crossover between the *OR* repeats. Ectopic recombination at these sites can lead to a dicentric intermediate which on breakage can lead to a duplicated chromosome 8p, the inv dup(8p). The complement of this breakage event was speculated to be a terminal deleted chromosome del(8p).

In this paper we describe a girl with a mosaic del(8p)/inv dup(8p) in blood lymphocytes. Mosaics with two cell lines carrying two different rearranged chromosomes are extremely rare. Two reports describe the presence of a mosaic with the same chromosome deleted in one cell line and the same chromosome duplicated in another cell line.[9 10] The most straightforward explanation for the finding of two cell lines in the blood of this patient carrying either a chromosome 8p+ or a chromosome 8p– would be the following postzygotic series of events. A sister chromatid exchange formed an aberrant U type chromosome 8, which at anaphase yielded an unstable dicentric chromosome 8 in one of the daughter cells. The unstable dicentric chromosome was pulled towards two different poles during a subsequent metaphase causing a break in 8p, thus resulting in a cell line with 8p+ and a cell line with an 8p– chromosome.[9 10] In contrast to this simple model, we show here that the 8p+ chromosome originated during maternal meiosis by ectopic recombination at the *OR* gene clusters by the mechanism described by Floridia *et al*[1] and that the 8p– chromosome originated by an independent terminal deletion event.

Key points

- We describe a mentally retarded girl with a mosaicism 46,XX,del(8p)/46,XX,inv dup(8p) in her lymphocytes and a single cell line with 46,XX,inv dup(8p) in her fibroblasts.
- Rather than being the product of an intermediate dicentric chromosome that became broken and generated both the 8p+ and the 8p– chromosomes, we show that both aberrant chromosomes were generated independently. The inv dup(8p) chromosome originated during maternal meiosis by ectopic recombination at the *OR* gene clusters. The 8p– chromosome resulted from a terminal deletion event, either by a mitotic error from the inv dup(8) or by a meiotic error from the second sister chromatid.
- Molecular analysis of the inv dup(8p) refines the models that describe the mechanism of origin of the inv dup(8p) and adds to the notion that many mosaics may be of meiotic origin.

CASE REPORT

The patient, a girl, was the first and only child of young, healthy parents of Turkish origin. Their family histories were normal without consanguinity.

Pregnancy, and delivery at 39 weeks, were normal. Birth weight was 2470 (3rd centile), length 45.5 cm (3rd centile), and head circumference 33.5 cm (10th centile). Feeding problems with gastro-oesophageal reflux were present from the beginning. Psychomotor development was retarded and at the age of 13.5 months motor and cognitive development were moderately retarded (developmental level of 8 months on the Bayley developmental scale). At the age of 15 months she was referred to the genetic clinic for further diagnostic evaluation. Weight was 7.2 kg (3rd centile 7.8 kg), length 70 cm (3rd centile 72.5 cm), and head circumference 46.5 cm (50th centile). Facial appearance was mildly dysmorphic with upward slanting palpebral fissures, synophrys, and left preauricular tag. Both thumbs were low set with hypotrophic thenars, and there was bilateral clinodactyly of the fifth fingers. Except for manifest axial hypotonia, neurological findings were normal (fig 1). The most striking finding was the presence of linear areas of depigmentation with bordering areas of hyperpigmentation on the lumbar and presacral region and on both legs, most evident on the medial sides of both thighs without signs of body asymmetry. Now, at the age of 2 years,

Abbreviations: FISH, fluorescence in situ hybridisation; FITC, fluorescein-isothiocyanate

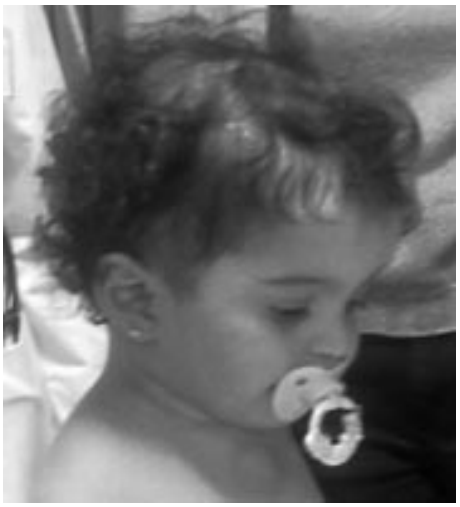

Figure 1 Photograph of the child at the age of 2 years.

psychomotor development is more severely retarded than estimated at the age of 18.5 months (motor developmental level of 10 months and cognitive level of 13 months, Bayley developmental scale).

Routine biochemical screening, cardiac and renal echographies, and computed tomography of the brain were normal.

MATERIALS AND METHODS

Cytogenetic studies

High resolution G banded chromosomes were prepared from peripheral white blood cells, according to standard procedures.

Fluorescence in situ hybridisation (FISH)

Polymorphic markers were used as a reference to isolate BACs 16G2 (D8S1819), 211C9 (D8S1672), 375N15 (D8S516), 999I21 (D8S1477), 22F19 (D8S1769), and 910E6 (D8S283) from the ENSEMBL Golden Path.[4] PAC 1043K1 and YACs 799B1, 859A7, 770C2, and the 8p subtelomeric probe 580L5 have been described.[2 11] Probes were constructed by isolating genomic DNA from BACs using Nucleobond AX100 (Machery-Nagel). One microgram of DNA was labelled with either biotin-16-dUTP (Boehringer Mannheim) by the nick translation system (Invitrogen) or by digoxygenin using the DIG Nick mix (Roche). Unincorporated nucleotides were removed with the QIAquick Nucleotide removal kit (Qiagen). Before FISH, cells were air dried on slides and pretreated with pepsin, followed by denaturing in a 70% formamide/2 × SSC (0.15 mol/l NaCl, 0.03 mol/l Na_3citrate; pH 7) solution at 72°C and subsequently dehydration with ethanol. FISH was performed as described.[2] After hybridisation O/N at 37°C, the slides were washed for one minute in 0.4 × SSC/0.3% NP40 solution at 72°C, one minute at 2 × SSC/0.1% NP40 solution at RT, and three minutes in 4T (4 × SSC; 0.05% Tween 20; pH 7.0). Slides were subsequently incubated with blocking buffer (4 × SSC; 0.05% Boehringer Blocking reagent) for 20 minutes at 37°C, 100 µl (20 µg/ml in 100 ml 4BB) of fluorescein-isothiocyanate (FITC) labelled avidin (Vector Laboratories) was added and hybridised for 30 minutes at 37°C, washed twice for four minutes in 4T (4 × SSC; 0.05% Tween 20; pH 7.0), and four minutes in TNT (100 mmol/l Tris (HCl); 150 mmol/l NaCl; Tween 20; pH 7.0), 100 µl of a mixture biotinylated of antiavidin (5 µg/ml) (Vector Laboratories)/ rhodamine labelled sheep antidigoxygenein (2 µg/ml) (Boehringer) and hybridised for 30 minutes at 37°C, washed three times for four minutes in 4TNT, and finally incubated with FITC labelled avidin and texas red labelled donkey antisheep (Jackson Laboratories) and hybridised for 30 minutes at 37°C.

Figure 2 Partial G banded karyotype showing the normal chromosome 8 and the del(8) on the left and a normal chromosome 8 and the inv dup(8) on the right. Ideograms of the normal and the derivative chromosomes 8 are shown.

The slides were washed three times for four minutes in TNT, five minutes in 1 × PBS, and dehydrated in an ethanol series of 70%, 90%, and 100%.

The cells were counterstained with DAPI and the slides were mounted in Vectashield mounting medium (Vector Laboratories). The signal was visualised by digital imaging microscopy with Cytovision capturing software (Applied Imaging).

FISH analysis was also used for the study of the inversion polymorphism. To this end, FISH was performed with clones 375N15 and 1043K1, both of which lie inside the inverted region, and 16G2, distal to the inverted region. Nuclei where the three probes occurred close to each other and in a linear arrangement to exclude possible misalignment were examined. One hundred interphases were scored in each parent.

Molecular analysis

CA repeats spaced along the short arm of chromosome 8 were amplified by polymerase chain reaction using a FITC label on one of the primers. The fragments were sized on an ALF DNA sequencer (Amersham Pharmacia Biotech). Primer sequences were as published.[12]

RESULTS

Cytogenetic analysis

Cytogenetic analysis showed the presence of two cell lines in equal ratios in the blood lymphocytes: 46,XX,del(8)(p23.3) inv dup(8)(p21.1p23.2)/46,XX,del(8)(p21.1). In skin fibroblasts only a single cell line 46,XX,inv dup(8p) was found. The chromosomes of the parents were normal. A partial karyotype showing the aberrant chromosome 8 is depicted in fig 2.

To define the regions duplicated and deleted on the derivative chromosomes 8, a series of BAC probes was hybridised on metaphase spreads of the patient lymphocytes and investigated by FISH (figs 3 and 4). On the inv dup(8p) chromosome the probes located distal to the *OR* gene cluster (580L5 and 16G2) are deleted, the probes from between the two clusters (211C9, 375N15, and 1043K1) are present in a single copy, and probes proximal to the second *OR* gene cluster are duplicated (YACs 799B1 and 859A7, BACs 999I21 and 22F19). On the del(8p) chromosome probes, 799B1 and 859A7 were not hybridising while probes 999I21, 22F19 were present. Finally, the most proximal probes 910E6 and YAC 770C2 were present in single copy on both the 8p+ and 8p− chromosomes. In conclusion, the breakpoint on the del(8p) chromosome is between YAC 859A7 and BAC 999I21. The duplication on the inv dup(8p) extends from the *OR* to between BAC 22F19 and BAC 910E6.

Molecular analysis: ectopic recombination originated during maternal meiosis

To investigate the origin of the mosaicism and confirm the FISH analysis, polymorphic marker analysis was performed

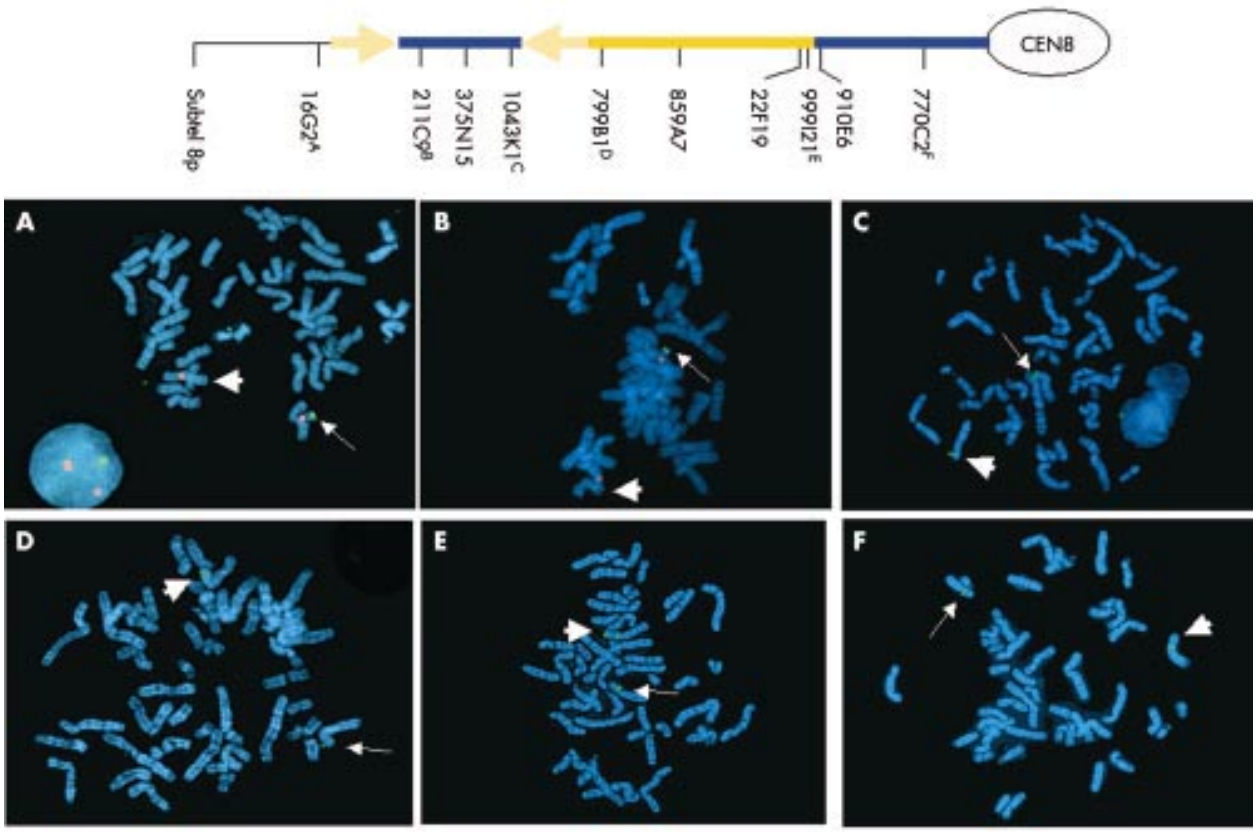

Figure 3 FISH analysis showing a series of probes hybridised on the inv dup(8p). (A) Schematic representation of the location of the probes. The blue line represents the chromosomal region present as a single copy, the yellow region the duplicated chromosomal region, and the thin black line the deleted region. The arrows represent the *OR* gene clusters. Probes are indicated below the line, the superscript letter indicating the plate in panel B. (B) FISH pictures with the respective probes. All probes are visualised in green. In plates A, B, and D a centromere 8 specific probe has been hybridised and visualised as a red signal.

on DNA isolated from lymphocytes and from skin tissue culture cells of the patient as well as on the DNA isolated from the lymphocytes of the parents (table 1).

Markers D8S264 and D8S1781 show that only a single paternal allele was detected in the region distal to the *OR* cluster (fig 5A). For marker D8S520, located between the *OR* gene clusters, a single paternal and maternal allele can be seen in both blood and fibroblasts (fig 5B). In blood, the peak of the maternal allele can be detected at about half the peak height seen in fibroblasts. This is as expected since half of the cells in blood contain del(8p).

Markers D8S552 and D8S261 show the presence of the paternal allele and two maternal alleles in both blood and fibroblasts (fig 5C). As expected, also here in blood the maternal allele peak height is about half the peak height found in fibroblasts. This confirms that this region is duplicated in the inv dup(8p) and absent in the del(8p) chromosome.

Markers D8S1820, D8S1769, D8S1810, and D8S1477 also show the presence of the paternal allele and two maternal alleles in both blood and fibroblasts. Peak intensities in the fibroblasts were also higher than in blood (fig 5D). However these peak intensities were not in a one to one ratio but in a two to one ratio. This indicates the presence of three maternal copies in the blood. This confirms the FISH finding that BACs containing these polymorphic markers are duplicated on the inv dup(8p) and present in a single copy on the del(8p).

Finally, the most proximal markers D8S283 and D8S532 as well as markers on 8q (D8S529, D8S553, and D8S1836) show the presence of only a single paternal and maternal allele (data not shown). The peak intensities of the maternal allele are equal in both fibroblasts and blood, confirming that this region is present on both inv dup(8p) and del(8p) in a single copy.

Inversion heterozygosity at the 8p– *OR* gene cluster in both parents

To investigate whether either a meiotic recombination event among the *OR* gene clusters could have been stimulated by the presence of an inversion heterozygosity at the 8p *OR* gene cluster in the chromosomes of one of the parents, interphase FISH was performed on lymphocytes from both parents. Using combinations of probes in two colour interphase FISH experiments (with two probes from within the common deletion and one from outside), we determined the orientation of the region between the 8p– *OR* gene cluster. In the interphase nuclei of the mother, 52% of the signals had a yellow-red-green, 33% a red-yellow-green, and 14% a yellow-green-red orientation. In the interphase nuclei of the father, 52% of the signals showed a yellow-red-green, 37% a yellow-green-red, and 11% a green-yellow-red orientation (fig 6). That slightly more signals were counted with the yellow-red-green than with the red-yellow-green orientation may be caused by a difference in distance among the probes when normal versus inverted. This result makes us conclude that both parents were heterozygous for the 8p23.1 inversion polymorphism.

DISCUSSION

Constitutional chromosomal mosaicism with two cell lines carrying two different rearranged chromosomes which cytogenetically balance each other was thought to be the result

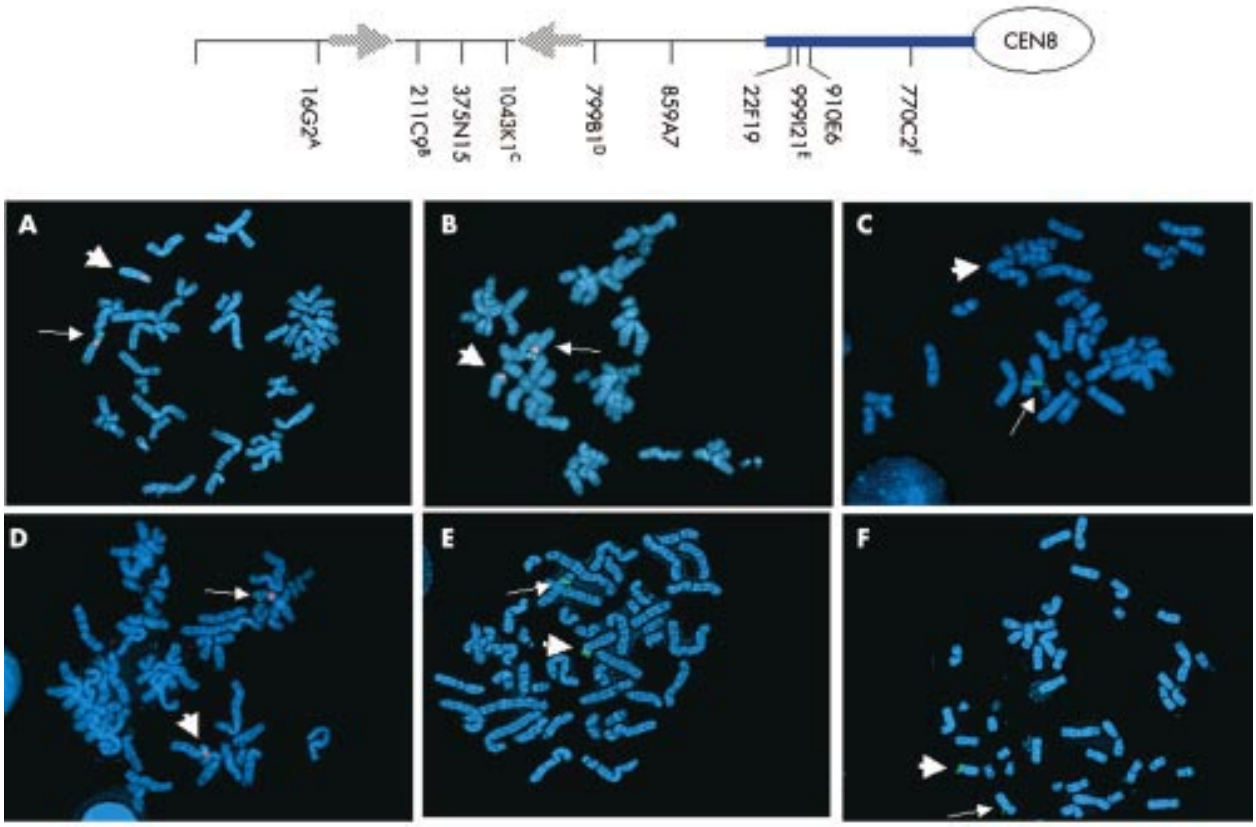

Figure 4 FISH analysis showing a series of probes hybridised on the del(8p). (A) Schematic representation of the location of the probes. The blue line represents the chromosomal region present as a single copy and the thin black line the deleted region. The arrows represent the *OR* gene clusters Probes used in the analysis are indicated below the line, the number indicating the plate in B. (B) FISH pictures with the respective probes. All probes are visualised in green. In plates 1, 2, and 4, a centromere 8 specific probe has been hybridised and visualised as a red signal.

Table 1 Results of the polymophic marker analysis

			Allele values			
Marker	Position	Location (UCSC)	Father	Mother	Child fibroblasts	Child blood
D8S264	8p23.3	1.811	126/138	126/136	126	126
D8S1781	8p23.2	3.545	249/263	249/255	249	249
D8S520	8p23.1	10.519	178/192	194/198	192/198	192/198
D8S552	8p22	12.346	176/180	178/182	178/180/182	178/180/182
D8S261	8p22	17.602	130/132	128/130	128/130/132	128/130/132
D8S1820	8p21.1	27.680	104/114	112/116	104/112/116	104/112/116
D8S1810	8p12	31.496	178/192	192/194	178/192/194	178/192/194
D8S1769	8p12	31.496	243	245	243/245	243/245
D8S1477	8p12	31.871	140/176	144/165	140/144/165	140/144/165
D8S283	8p12	33.43	115	115/121	115/121	115/121
D8S532	8p11.21	40.138	245/251	243/253	251/253	251/253
D8S553	8q13.1		217	242/244	217/242	217/242
D8S529	8q24.22		240/244	236/242	240/242	240/242
D8S1836	8q24.3		133/147	131/135	135/147	135/147

of a post-fertilisation mitotic error.[9 10] In contrast to this assumption, we showed that in this patient a rearrangement occurred during the female meiosis generating the 8p+ chromosome and that the 8p– chromosome resulted from an independent terminal deletion event.

The 8p+ chromosome results from an ectopic recombination event within the 8p *OR* gene clusters. Our findings substantiate the models of Floridia *et al*[1] and Giglio *et al*.[4] Probes and markers distal to the telomeric gene cluster were absent on the 8p+ chromosome, probes and markers between the *OR* genes were present in a single copy, and probes and markers centromeric to the *OR* gene clusters up to D8S1477 were duplicated in the inv dup(8p). That the rearrangement is caused by ectopic recombination at the *OR* gene cluster and not at a nearby site is substantiated by the finding that BAC 16G2 which flanks the distal *OR* gene cluster is absent whereas YAC 799B1 which flanks the proximal *OR* gene cluster is duplicated on the inv dup(8p). Both parents carry the inversion polymorphism between the *OR* gene clusters at 8p23.1 supporting the thesis that this inversion polymorphism causes susceptibility for this rearrangement. The polyporphism has been detected in all mothers of children with the inv dup(8p) analysed so far.[4]

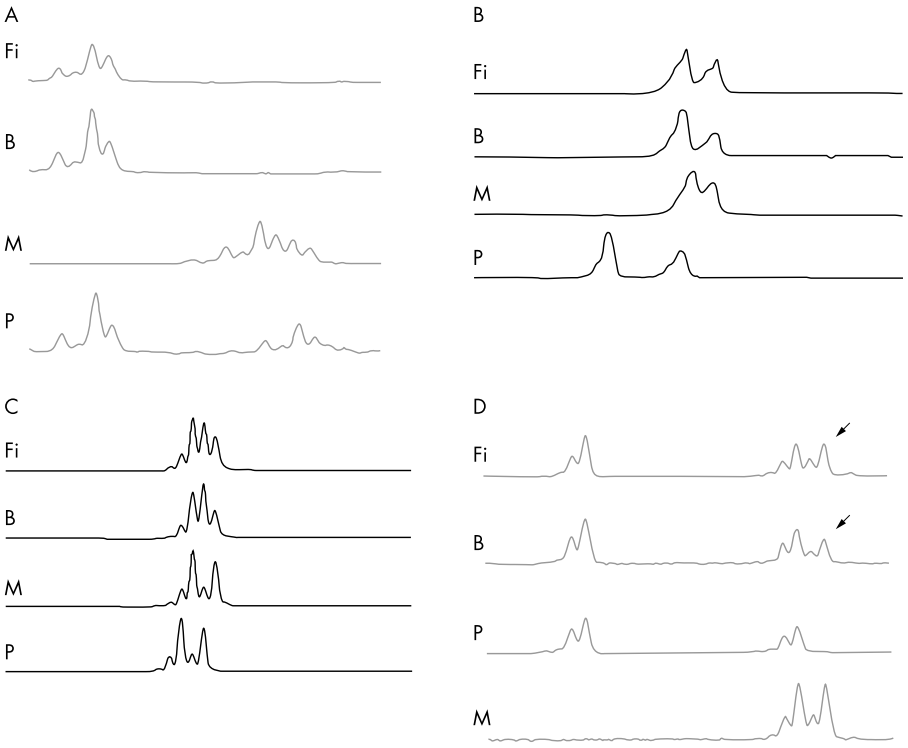

Figure 5 Polymorphism analysis on D8S1781 (A), D8S520 (B), D8S552 (C), and D8S1810 (D) on DNA extracted from fibroblasts (Fi), blood (B) of the patient, blood of the mother (M), and father (P). The arrow in panel D indicates the peak deviating from a one to one ratio indicating that two copies of this maternal allele are present.

Marker analysis indicates that the inv dup(8p) is partially heterodisomic showing that both maternal homologous chromosomes are involved. This has been found previously and explained by two models: (1) an intersister chromatid recombination event took place at the *OR* gene cluster and two more recombination events occurred on the short arm of chromosome 8.[3] However, this model would imply that three crossovers occur at short distances which, on theoretical grounds, would be excluded by crossover interference. Moreover, this model would predict that a small region below the *OR* gene cluster would be isodisomic, which was not found in our patient. (2) An interhomologous chromatid ectopic recombination event at the *OR* gene cluster took place,[1] which at anaphase I results in a dicentric isochromosome that undergoes a breakage at one centromere or between centromeres, owing to the instability caused by the presence of two centromeres. Our data favour this model.

Floridia *et al*[1] hypothesised that the same mechanism which causes the inv dup(8p) rearrangement might also account for some of the 8p deletions. The detection of both the inv dup(8p) and the large terminal deleted chromosome del(8p) in this patient led us firstly to assume that this 8p– was the complement of the 8p+ derived after breakage of an intermediate dicentric chromosome at anaphase I. Contrary to this assumption, we discovered that the del(8) contains sequences which are duplicated on the inv dup(8p). Hence, the del(8) cannot be the mere counterpart of the broken dicentric chromosome. We can envision three scenarios by which the del(8) has arisen. (1) A terminal deletion of the inv dup(8p) occurred in a somatic cell during development thus generating a secondary cell line containing the del(8p). However, it is unclear how the deletion would have been rescued. Healing of broken chromosomes by de novo telomere synthesis has been shown to occur only in the germline and not in somatic tissues unless immortalised.[13–15] In somatic tissues healing of broken ends occurs by capturing the telomere from another chromosome.[16] However, we have no cytogenetic evidence for the presence of another chromosomal fragment on the del(8). (2) A terminal deletion occurred in the second bivalent and in addition non-disjoined chromatid during anaphase II. A sperm fertilised the disomic 8p+/8p– egg. During development two independent non-disjunction events took place generating a cell line containing the 8p+ chromosome and a cell line containing the 8p– chromosome. (3) The dicentric chromosome 8 was inherited by the zygote. Dicentric chromosomes 8 have been reported in patients.[17 18] Most often one or both centromeres is inactivated. Two separate breakage events of the dic(8) could have created a cell line containing the inv dup(8p) and another cell line with del(8p).

There are very few reports of mosaicism with the two cell lines carrying 46 chromosomes. Because of the simplicity by which a rearranged chromosome can be invoked, it is often assumed that rearranged chromosomes in mosaics originate during mitosis. Possibly because of this assumption, the origin of the rearrangement has only been investigated in seven reports and in none do the two cell lines carry different aberrant chromosomes.[19–25] In three reports a meiotic error occurred first, followed by either a loss or a rearrangement of the trisomic chromosome.[19 22 24] The finding in this patient reinforces the idea that many chromosomal rearrangements occurring in mosaic people may have their origin during meiosis.

Interestingly, the patient has only a mild phenotype. Except for hypotonia and mental retardation, none of the features regularly seen in patients with inv dup(8p) have been found. These features include brachycephaly, distinct facial appearance with prominent lips, joint contractures and/or hyperextensibility, and frequent occurrence of cardiac defects.[7] Similarly, the patient did not have features typical for patients with deletions of 8p. No patients with del(8)(pter→p12) have been described and such deletions are probably incompatible with life.[7]

The presence of both 8p+ and 8p– cell lines in the blood and possibly in other tissues seems sufficient to compensate for

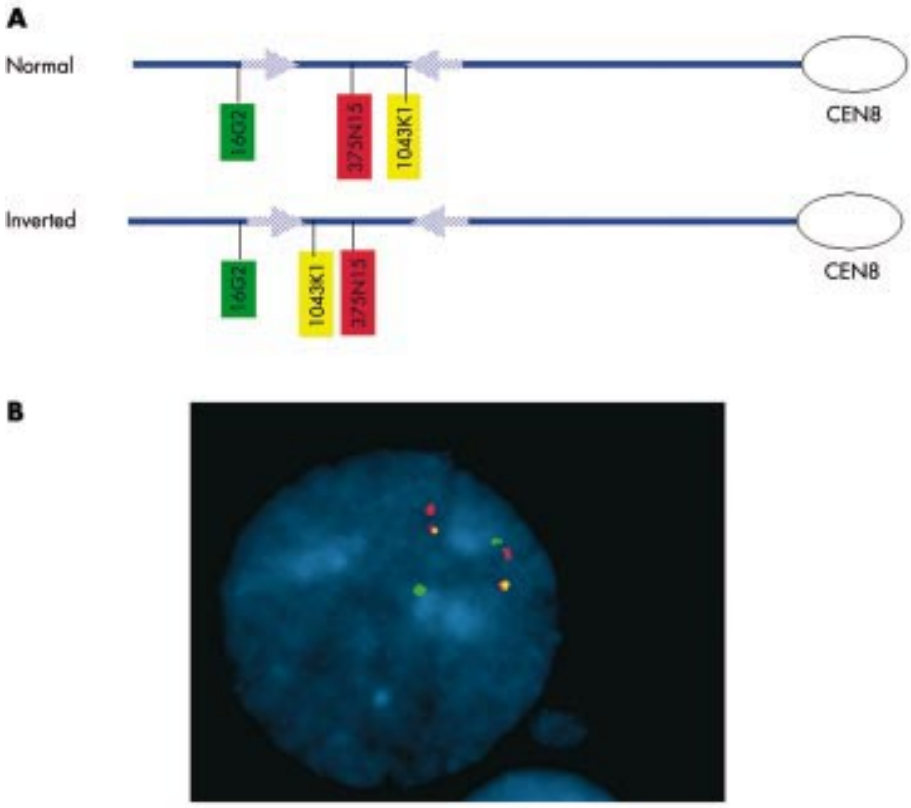

Figure 6 (A) Schematic map of 8p showing the commonly deleted region on 8p. This is not drawn to scale. The yellow arrow boxes indicate the location of the *OR* gene clusters. The probes used for FISH analysis with their respective colour codes are indicated underneath. (B) Dual colour FISH on interphase cells showing the regular alignment in one chromosome and the inversion polymorphism on the other chromosome.

the detrimental effects of either the sole presence of inv dup(8p) and/or del(8p). In patients with a deletion of 8p23.1 commonly heart defects and behavioural problems are found.[2 3 5] Because no heart defects were found in this patient, the mosaic presence of GATA4 seems to compensate for the effects of GATA4 haploinsufficiency.

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