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ARTICLE

Mosaic 22q13 deletions: evidence for concurrent mosaic segmental isodisomy and gene conversion

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Although 22q terminal deletions are well documented, very few patients with mosaicism have been reported. We describe two new cases with mosaic 22q13.2-qter deletion, detected by karyotype analysis, showing the neurological phenotype of 22q13.3 deletion syndrome. Case 1 represents an exceptional case of mosaicism for maternal 22q13.2-qter deletion (45% of cells) and 22q13.2-qter paternal segmental isodisomy (55% of cells). This complex situation was suspected because cytogenetic, FISH and array-CGH analyses showed the presence of an 8.8 Mb mosaic 22q13.2-qter deletion, whereas microsatellite marker analysis was consistent with maternal deletion without any evidence of mosaic deletion. Molecular analysis led to the definition of very close, but not coincident, deletion and uniparental disomy (UPD) break points. Furthermore, we demonstrated that the segmental UPD arose by gene conversion in the same region. In Case 2, mosaicism for a paternal 8.9 Mb 22q13.2-qter deletion (73% of cells) was detected. In both patients, the level of mosaicism was also verified in saliva samples. We propose possible causative mechanisms for both rearrangements. Although the size of the deletions was quite similar, the phenotype was more severe in Case 2 than in Case 1. As maternal UPD 22 has not been generally associated with any defects and as the size of the deletion is very similar in the two cases, phenotype severity is likely to depend entirely on the degree of mosaicism in each individual.
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Introduction

To date, thanks to the increasing use of subtelomeric FISH tests, more than 100 cases with monosomy 22q13.3 have been reported. Among these, very few patients showed mosaicism for the 22q13.3 deletion; in fact, the majority of mosaic 22q13 deletions is the product of ring chromosome 22 or of a more complex structural chromosome 22 aberration involving a chromosome deletion and an inverted duplication combined with ring chromosome formation.[1–6]

We report two new cases of mosaicism for the del(22)(q13.2) diagnosed by routine conventional high-resolution G-banded karyotype and refined by array CGH analysis. In one case, molecular characterization revealed additional mosaic segmental uniparental disomy (UPD) in

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the 'normal' cell line. Both patients showed all the main features of 22q13.3 deletion syndrome, suggesting that *SHANK3* gene haploinsufficiency[7–10] is sufficient to express the neurological phenotype of the syndrome even in mosaic.

Methods

Two patients with mosaic 22q13 deletion were ascertained through routine karyotype analysis. Clinical details are provided as Supplementary files.

FISH experiments were performed with the ARSA probe (Vysis) and 22q-cosmid clones n66c4, n85a3, n94h12, and n1g3 as previously reported.[11] Array-based CGH was performed using the Agilent Human Genome CGH Microarray Kit 44B as previously described.[12]

Genomic DNA was extracted from probands' blood, EBV cell lines, parents' blood, and saliva with standard protocols. Somatic cell hybrid clones were generated as in Giorda *et al*.[13]

The UCSC Genome Browser (May 2004 assembly; http://genome.ucsc.edu/) maps and sequence were used as references. Genotyping of polymorphic sequence-tagged sites was performed by amplification with primers labeled with fluorescent probes followed by analysis on an ABI 310 Genetic Analyzer (Applied Biosystems). Amplifications were performed with AmpliTaq Gold (Applied Biosystems) using standard protocols.

Further genotyping was performed using the Illumina HumanHap500 single beadchip with 750 ng of genomic DNA at a standard concentration of 50 ng/ml. The process conformed to the manufacturer's protocols. The Illumina Beadstudio algorithm was used to call genotypes with manual analysis to confirm cluster boundaries.

Long-range PCRs were performed with JumpStart Red ACCUTaq LA DNA polymerase (Sigma). Fragments were cloned in pCR-4 TOPO using a TOPO TA cloning kit (Invitrogen). All sequencing reactions were performed with a Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) and run on an ABI Prism 3130xl Genetic Analyzer.

For Case 1 break point cloning, two chromosome 22-specific primers adjacent to the deletion break points were designed and used for nested ACP-PCR.[11] PCR products were both directly sequenced and cloned, followed by sequencing of individual clones.

Dilution cloning was performed by seeding 96-well plates with cells from Case 1 EBV line diluted to 0.3 cells per well in RPMI medium containing 10% FCS. DNA was extracted from the resulting clones with DNAzol.

Results

Clinical characteristics

Clinical features are summarized in Table 1. As in non-mosaic 22q13 deletion, both patients showed all the main features of 22q13.3 deletion syndrome (global developmental delay, delayed speech, accelerated growth, neonatal hypotonia, minor dysmorphism, and autistic-like behaviour). The psychomotor delay was more severe in Case 2 than in Case 1.

Cytogenetic analysis revealed in both cases a *de novo* mosaic 22q13.2-qter deletion.

The karyotype was as follows:

Case 1: 46,XX,del(22)(pter→q13.2::)[45]/46,XX [55].

Case 2: 46,XX,del(22)(pter→q13.2::)[73]/46,XX [27].

FISH analysis with ARSA and 22q cosmid probes confirmed a pure terminal 22q13 deletion in both cases. In particular, in Case 1 we also found one metaphase, out of 200 examined, containing three non-deleted chromosomes 22.

Array CGH analysis demonstrated that the deletion encompasses the terminal 8.8 Mb (Figure 1a) and 8.9 Mb (Figure 1b) in Case 1 and in Case 2, respectively.

Typing of polymorphic chromosome 22 markers in the deleted interval in the probands and their parents demonstrated the following:

Case 1: the 22q13 deletion was of maternal origin without any evidence of mosaic deletion (Figure 1c and Table 2).

Case 2: the 22q13 deletion involved the paternal chromosome 22, with evidence of deletion mosaicism (Figure 1d and Supplementary Table 1).

Somatic cell hybrids, break point cloning, and sequencing

As in Case 1 cytogenetic investigations had clearly demonstrated a mosaic 22q13.2-qter deletion, whereas microsatellite analysis showed a maternal deletion without any evidence of mosaicism, the presence of segmental paternal isodisomy in the 'normal' cell line was supposed. We tested this hypothesis on somatic cell hybrid clones made by fusing Case 1's lymphoblastoid line with a Hamster cell line.

We isolated and analyzed 76 clones with informative chromosome 22 markers. We found three clones with a normal paternal chromosome 22, one with a deleted maternal 22, one with a non-deleted maternal chromosome 22 with segmental paternal UPD of its distal portion (Table 2).

We mapped and cloned with ACP-PCR the break point of the deleted chromosome 22. The deletion break point is followed by a TT dinucleotide and a series of $(AGGGTT)_n$ telomeric repeats, and it localizes approximately in the middle of a 1.3 kb $(GAAA)_n(GAAAAA)_n$ simple repeat in intron 5 of the *CENPM* gene (Figure 2a, Supplementary Figure 1). The repeat was very unstable when cloned in bacteria and generated clones containing a variety of deletions. This probably explains why the current Genome Map shows it as a 131 bp sequence (chr22:40665726–40665857).

Table 1 Summary of the clinical features of mosaic 22q13 deletion cases

Patients	*Case 1*	*Case 2*	*Case 3, Yong et al,*[18]
Age at diagnosis	5 years	2 years 8 months	5 years 6 months
Sex	Female	Female	Female
Mother'age at birth (years)	36	38	36
Father's age at birth (years)	31	42	43
Perinatal			
Gestational weeks at birth	38	39	37
Weight at birth (g)	2490 (25th)	3300 (50th)	3225 (50th–90^{th}))
Length at birth (cm)	49 (75th)	49 (10th)	51 (90th)
Head circumference at birth (cm)	34 (75th)	35 (10th)	31.5 ($<10^{th}$)
Apgar score at 1′ and 5′	9/9	9/10	9/9
Neurodevelopment and CNS			
Hypotonia	+ (neonatal)	+ (since the first months of life and still present)	Not reported
Global developmental delay	+ (moderate)	+ (severe)	+ (severe)
Delayed/absent expressive language	+	+	+
Epilepsy/seizures	Rare febrile convulsions	Absence seizures in the first year of life	Atonic fits/secondary generalized epilepsy
Autistic behaviour	+	+	Not described
Walking	Gait ataxia	Unable to walk at $3^{6/12}$ years	Unsupported walking at 20 months
EEG	Normal	Normal	Continuous generalized slow waves and multiple foci of sharp waves in both hemispheres.
Cerebral MRI	Cavum vergae and large septum pellucidum cysts	Moderate enlargement of lateral ventricules and of hemispheric subarachnoid spaces; delayed myelinization pattern	Not reported
Growth			
Normal/accelerated growth	+	+	−
Advanced skeletal age	+	+	Not studied
Dysmorphisms and malformations			
Dolichocephaly	+	−	−
Epicanthic folds	+	+	+
Bulbous nose/wide nasal bridge	+/+	−/+	+
Mandibular abnormalities	Pointed chin	Micrognathia	−
Large/dysplastic ears	+	+	+
Fingers abnormalities	Arachnodactyly	Tapering fingers with hypoplastic distal phalanges and ulnar deviation of the second fingers	Not reported
Other features	Decreased sensitivity to pain; pectus excavatum	Ano-cutaneous fistula, mild left hydronephrosis	Abnormal hyperpigmentation on the left cheek and lower limbs

The proband's non-deleted maternal chromosome 22 carries the maternal allele at four polymorphisms (1226–1246) located downstream of the deletion break point (Supplementary Figures 1 and 2A–F, left).

SNP analysis of the proband and her parents with an Illumina HumanHap500 Infinium 2 chip confirmed the segmental UPD-22 and positioned its break point between SNPs rs1022533 (chr22:39302493) and rs1062753 (chr22:40722757), but SNP rs10483213 (chr22:40669471) showed unexpected results (Supplementary Table 2). Sequencing analysis demonstrated that in a subset of the cells the paternal chromosome 22 carried the maternal rs10483213 allele (Table 2, Supplementary Figure 2A–F, right). This result is consistent with the occurrence of a gene conversion event. Complete sequencing of the chromosome 22 segment between 40664700 and 40678040 in all family members revealed an additional polymorphism, 22-5(G/A) at Chr22:40676675, informative for paternal UPD (Table 2). These data demonstrate that deletion and segmental UPD break points, while very close, do not coincide. The deletion break point is located between chr22:40665726 and 40665857; the segmental

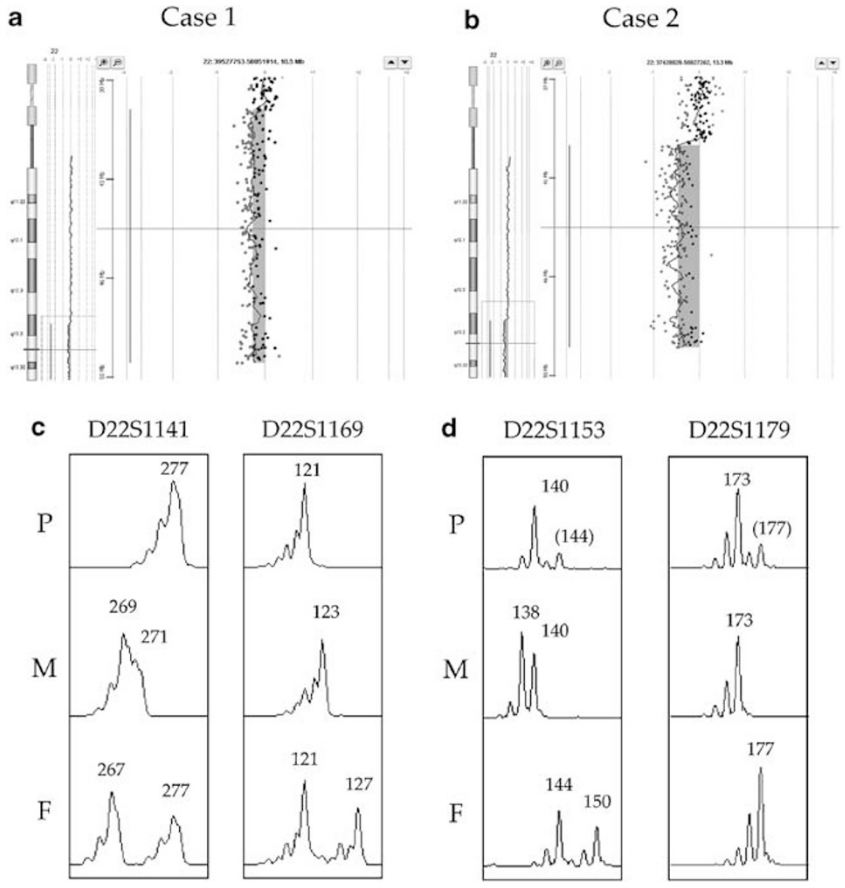

Figure 1 (**a** and **b**) (left), array CGH profile of chromosome 22 showing the deletion at 22q13.2-qter in Case 1 and Case 2, respectively. (**a**) (right), enlargement of Case 1 deleted region: the 22q-deletion break point lies between the oligomer at 40.659 Mb (still present) and the oligomer at 40.666 Mb (first deleted); probes from 40.666 to 49.468 Mb have an average $\log_2$ ratio of about −0.25, compatible with mosaic deletion; (**b**) (right), enlargement of Case 2 deleted region: the 22q deletion break point lies between the oligomer at 40.417 Mb (still present) and the oligomer at 40.494 Mb (first deleted); probes from 40.494 to 49.468 Mb have an average $\log_2$ ratio of about −0.44, compatible with mosaic deletion. (**c** and **d**) Parental origin of the 22q13 deletion in Cases 1 and 2, respectively. P, proband; M, mother; F, father. (**c**) Case 1: for both markers, the proband (P) has inherited only one paternal allele and no maternal alleles, indicating a deletion of maternal origin. Allele sizes are shown. (**d**) Case 2: the proband has inherited one maternal and one paternal allele in non-stoichometric proportions, indicating the presence of a mosaic deletion of paternal origin. The sizes of the mosaic deleted alleles are in commas.

UPD break point location can be narrowed down to a 7.2 kb region between rs10483213 and 22-5(G/A) (chr22:40669471–40676675) (Figure 2a).

To find out how the different maternal and paternal chromosomes 22 were distributed in the mosaic lines, we obtained pure EBV subclones containing either the deleted or the UPD-22 chromosome by dilution cloning. We typed all subclones for SNPs 1030–1050 and rs10483213, and for the telomere of the deleted chromosome 22. We found that the gene conversion carrying paternal chromosome 22 is associated with the maternal 22 with segmental UPD, while the paternal chromosome 22 that did not undergo gene conversion is associated with the deleted maternal 22 (Figure 2b and Supplementary Figure 2G–H).

In addition to blood, we also analyzed EBV lines and saliva from each proband.

We found in Case 1 the same degree of mosaicism in the proband's blood (not shown), EBV line, and saliva (Supplementary Figure 2A and I), whereas in Case 2 the level of mosaicism in different tissues seems to vary between 70 and 80% (Supplementary Figure 3).

Discussion

Apart from patients with r(22) chromosomes,[14–17] mosaicism for del(22)(q13) has so far been described in a 5.5-year-old girl with global developmental delay, epilepsy, and mild facial dysmorphisms,[18] and in two fetuses it was prenatally diagnosed because of ultrasound anomalies[19] and increased risk of Down's syndrome determined by maternal serum screening.[4] To the best of our knowledge, Case 1 represents the first patient with 22q13.3 deletion

Table 2 Molecular typing of Case 1

Locus	*Position (bp)*	*Blood DNA*			*Somatic cell hybrid clones*				*LD EBV clones*	
		Proband	*Mother*	*Father*	*wt 22-1*	*wt 22-2*	*UPD22*	*del(22)*	*del(22)*	*UPD22*
D22S280	31539000	213	213/215	213	213	213	213	213	NT	NT
D22S1162	32641000	157/151	151/161	157/163	157	157	151	151	NT	NT
D22S1158	32904000	219/237	237/219	219/235	219	219	237	237	NT	NT
1226 G/A	40665762	G/A	A/G	G	G	G	A	Null	G	G/A
1228 G/A	40665764	A/G	G/A	A	A	A	G	Null	A	A/G
1244 G/A	40665780	G/A	A/G	G	G	G	A	Null	G	G/A
1246 G/A	40665782	A/G	G/A	A	A	A	G	Null	A	A/G
rs10483213	40669471	A/G	G/A	A	A	G	G	Null	A	G
rs60025556	40673719	A	A/C	A	A	A	A	Null	NT	NT
rs8140869	40674243	A	A/G	A	A	A	A	Null	NT	NT
rs5996092	40674354	G	G/A	G	G	G	G	Null	NT	NT
22–5 G/A	40676675	G	A	G	G	G	G	Null	NT	NT
D22S418	41748000	141	135/141	141	141	141	141	Null	NT	NT
D22S1179	41922000	173	173/177	173/177	173	173	173	Null	NT	NT
D22S1141	44097000	277	269/271	277/267	277	277	277	Null	NT	NT
D22S1169	47788000	121	123	121/127	121	121	121	Null	NT	NT

Genomic DNAs was extracted from peripheral blood lymphocytes (blood), somatic cell hybrid clones containing isolated chromosomes 22, proband's EBV clones obtained by limiting dilution (LD). When two alleles are not in 1:1 ratio, they are printed in correspondingly larger/smaller size. Null, did not amplify; NT, not tested.

syndrome associated with a complex mosaic chromosome rearrangement consisting of a 22q13.2-qter chromosome deletion in 45% of her cells, and an apparently normal cell line that actually carries a 22q13 segmental paternal UPD in 55% of the cells.

Except for the possibility of homozygosity for recessive mutations, maternal UPD 22 does not seem to have an adverse impact on the phenotype.[20] This means that no maternally imprinted gene with major effect maps to chromosome 22. The phenotypic effects of paternal UPD 22 are still unknown, as no cases have been reported. Accordingly, our proband's clinical phenotype may or may not depend entirely on the presence and level of the mosaic 22q13 deletion. Psychomotor delay was more severe in Case 2 (73% of deleted cells in blood and over 80% in saliva) than in Case 1 (45% deleted cells in both blood and saliva). Similarly in the case reported by Yong,[18] with 95% of deleted cells, psychomotor delay was severe (Table 1). Whatever the level of mosaicism in the brain may be, it is enough to express the full neurological phenotype of the syndrome. Haploinsufficiency of the *SHANK3* gene is the main cause of the neurological phenotype in the 22q13 deletion syndrome.[7–10] Our cases demonstrate that even in mosaicism haploinsufficiency of *SHANK3* is sufficient to severely perturb its activity.

Mechanisms of mosaicism

As shown in Figure 2b, our cytogenetic and molecular analysis of Case 1 demonstrated the existence of a cell line with 22q13.2 maternal deletion and another with paternal isodisomy for the distal portion of chromosome 22q. The deleted cell line contains one normal paternal chromosome 22 and one maternal deleted chromosome 22; the UPD line contains one maternal chromosome 22 with its terminal 8.8 Mb derived from the paternal chromosome 22, and a paternal chromosome 22 carrying the maternal rs10483213 allele, evidence of mitotic interaction with a maternal chromosome 22. This gene conversion is most likely associated with the recombination event leading to paternal segmental isodisomy. The paternal chromosome 22 in the line carrying a deleted maternal chromosome 22 does not show any gene conversion (Supplementary Figure 2C and D).

Segmental isodisomy is usually assumed to be produced postzygotically by a mitotic exchange between non-sister chromatids. However, both maternal and paternal UPD should than be found in daughter cells, unless the mitotic event occurs very early and the products split between embryonic and extraembryonic tissues, or one of the products is lethal, as assumed in Beckwith–Wiedemann syndrome.[21] In our case, deletion and segmental UPD break points, while very close, do not coincide: on the UPD 22 chromosome, SNPs 1226–1246 and rs10483213, all distal to the deletion break point, contain a maternal allele (Figure 2a and Table 2). Fish analysis also demonstrated the presence of a cell with three copies of non-deleted chromosome 22. On the basis of these findings, we hypothesize two possible mechanisms leading to the formation of this complex mosaic.

In the first model (Figure 3a), a meiotic or post-meiotic event generated a deletion on the maternally inherited chromosome 22. During replication, one deleted chromatid repaired itself through break-induced replication[22,23] using one of the paternal chromatids as a template, generating a gene conversion during the process. This event resulted in the 55% Pat UPD + gene conversion

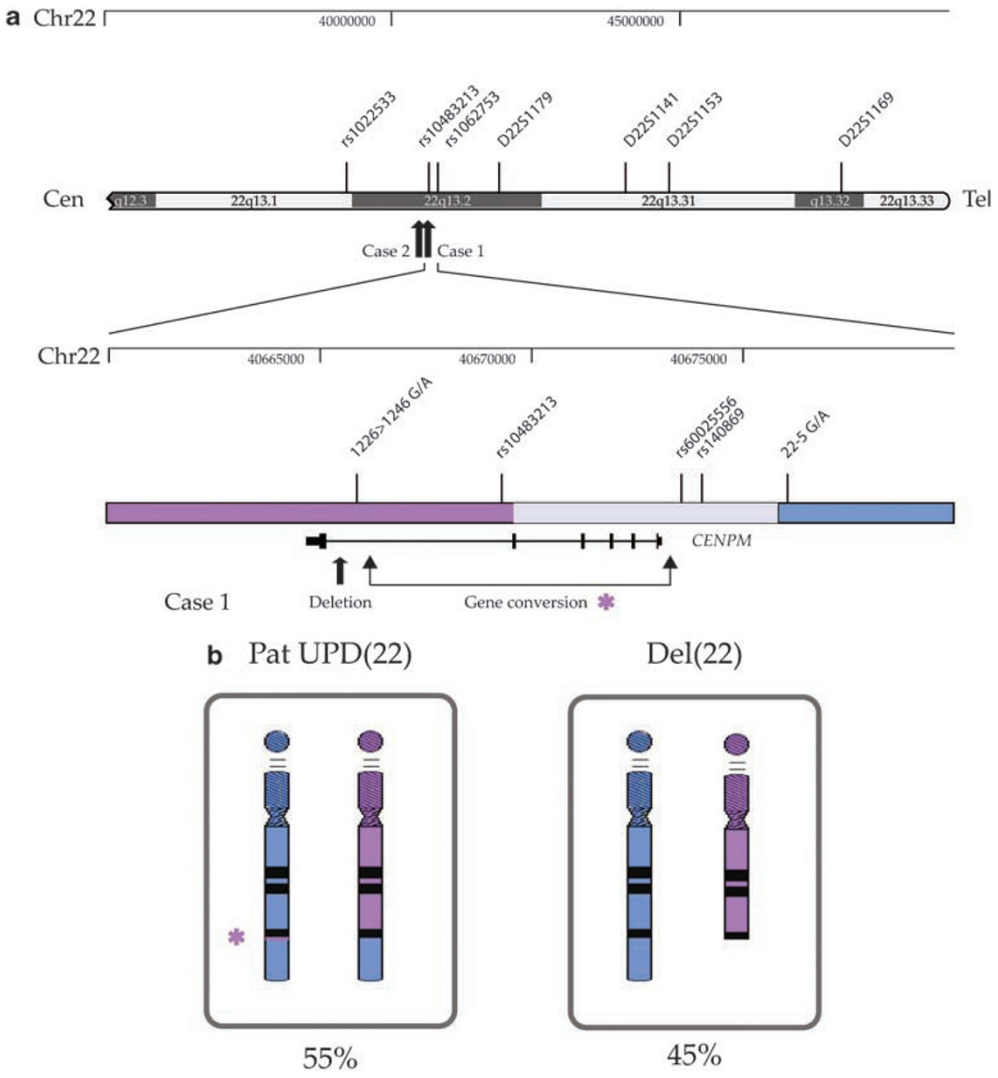

Figure 2 (**a**) Position of the break points. The distal 15 Mb of chromosome 22q are shown. The locations of cytogenetic bands and informative polymorphisms are indicated. The position of the break points in Cases 1 and 2 are marked by arrows. A blown-up detail of the 20 kb surrounding the Case 1 break point is also shown. Again, the locations of the *CENPM* gene, the deletion break point (arrow), and informative polymorphisms are indicated. The maternal portion of the chromosome is shown in pink, the paternal chromosome and segmental UPD in blue; the UPD break point region is in light blue. The upper limits of the gene conversion region are indicated by parentheses. (**b**) Graphic representation of the mosaic in Case 1. The two cell lines containing the deleted chromosome 22 [del(22)] or the 22q13 paternal segmental UPD [UPD(22)] are shown in gray frames. Paternal chromosome material is represented in blue, maternal material in pink. The gene conversion on one paternal chromosome 22 is indicated by a pink asterisk (*). The degree of mosaicism is also indicated.

lineage. The remaining replicated deleted maternal allele, still containing an unprotected chromosome end, lost several kilobases because of exonucleolytic activity. The secondary structure produced by the AT-rich repeat probably protected the chromosome from further degradation and facilitated stabilization by telomere healing. This is why the deletion break point of the maternal deleted chromosome 22 is located in the middle of a 1.3 kb AT-rich repeat.

In the second model (Figure 3b), based on FISH data showing the presence of one cell with three copies of non-deleted chromosome 22, the rearrangement occurred in a trisomic zygote with two paternal 22q and one maternal 22q chromosomes. A multiple recombination event involving all three chromosomes caused gene conversion, segmental UPD, and deletion; the deleted chromosome then lost several kilobases before being stabilized by the addition of telomeric sequences.

In Case 2, the presence of a normal cell line is, most likely, consistent with a postzygotic event, requiring a single hit to explain both the deletion and the mosaicism (Supplementary Figure 4).

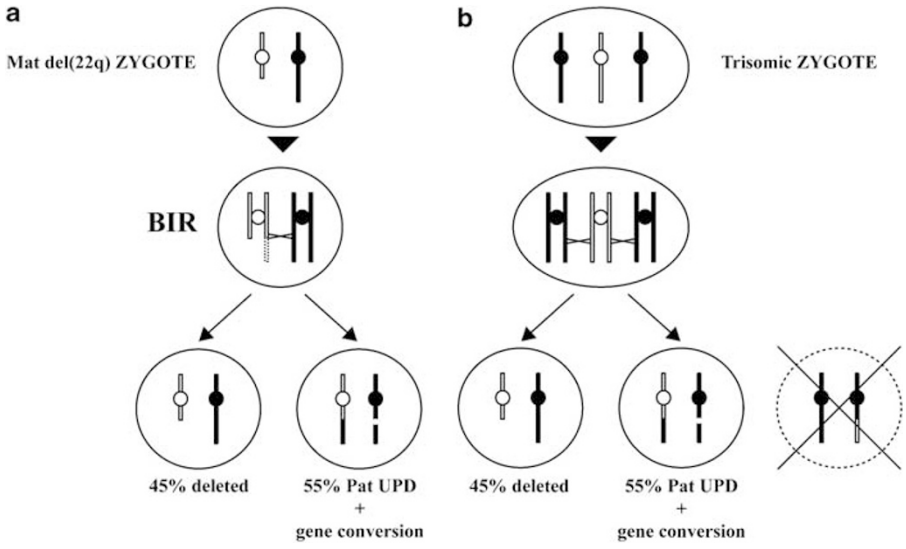

Figure 3 Mechanisms explaining the origin of the rearrangement in Case 1. (**a**) Rearrangement from a zygote containing a deleted maternal chromosome 22. After replication, one deleted replicated maternal allele recaptures the missing terminal portion of chromosome 22 from a paternal chromatid through BIR. During this maternal–paternal allele interaction, the paternal allele undergoes gene conversion. The products of this process form the 55% Pat UPD + gene conversion lineage. The other replicated deleted maternal allele loses several kilobases because of exonucleolytic activity (not shown), then is stabilized by telomere healing and forms the 45% deleted lineage. (**b**) Postzygotic rearrangement from a trisomic zygote. Multiple recombination involving two paternal and one maternal chromosomes 22 simultaneously generates gene conversion, isodisomy, and deletion. Paternal segmental UPD and gene conversion segregate in one cell line, deletion in another. We did not observe any cells with maternal segmental UPD. Paternal chromosome 22 is represented by a black-filled bar, maternal 22 by an empty bar.

A break during DNA replication in the zygote would generate a deleted chromatid, immediately stabilized by telomere healing. Segregation gave rise to a cell line with normal karyotype and another with 22q13 deletion.

In conclusion, we have described two patients with mosaic (45–75%) terminal 22q13 deletions, one of whom additionally carries a mosaic 22q13 segmental UPD (Case 1).

Keeping in mind that both cases showed the same 22q13 deletion size:

(1) the clinical phenotype, much more severe in case 2 than in case 1, should depend entirely on the presence of the mosaic 22q13 deletion; mosaic segmental UPD22 probably does not have relevant clinical impact.
(2) the complex mosaicism in Case 1 originated from a postzygotic or early embryonic recombination event causing a gene conversion event leading to the formation of segmental UPD and terminal deletion, both retained in mosaic in the fetus. Mosaicism in Case 2 was also probably due to a postzygotic event.

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References

1 Starke H, Mitulla B, Beensen V *et al*: First postnatal case of mosaic del(22)/r(22). *Prenat Diagn* 2003; **23**: 765–767.
2 Frizzley JK, Stephan MJ, Lamb AN *et al*: Ring 22 duplication/deletion mosaicism: clinical, cytogenetic, and molecular characterisation. *J Med Genet* 1999; **36**: 237–241.
3 Bergman A, Blennow E: Inv dup(22), del(22)(q11) and r(22) in the father of a child with DiGeorge syndrome. *Eur J Hum Genet* 2000; **8**: 801–804.
4 Phelan MC, Brown EF, Rogers RC: Prenatal diagnosis of mosaicism for deletion 22q13.3. *Prenat Diagn* 2001; **21**: 1100.
5 Lindquist SG, Kirchhoff M, Lundsteen C *et al*: Further delineation of the 22q13 deletion syndrome. *Clin Dysmorphol* 2005; **14**: 55–60.
6 Phelan MC, Rogers RC, Saul RA *et al*: 22q13 deletion syndrome. *Am J Med Genet* 2001; **101**: 91–99.
7 Bonaglia MC, Giorda R, Borgatti R *et al*: Disruption of the ProSAP2 gene in a t(12;22)(q24.1;q13.3) is associated with the 22q13.3 deletion syndrome. *Am J Hum Genet* 2001; **69**: 261–268.
8 Wilson HL, Wong AC, Shaw SR *et al*: Molecular characterisation of the 22q13 deletion syndrome supports the role of haploinsufficiency of SHANK3/PROSAP2 in the major neurological symptoms. *J Med Genet* 2003; **40**: 575–584.
9 Durand CM, Betancur C, Boeckers TM *et al*: Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet* 2007; **39**: 25–27.
10 Moessner R, Marshall CR, Sutcliffe JS *et al*: Contribution of SHANK3 mutations to autism spectrum disorder. *Am J Hum Genet* 2007; **81**: 1289–1297.

11 Bonaglia MC, Giorda R, Mani E *et al*: Identification of a recurrent breakpoint within the SHANK3 gene in the 22q13.3 deletion syndrome. *J Med Genet* 2006; **43**: 822–828.
12 Bonaglia MC, Marelli S, Gottardi G *et al*: Subtelomeric trisomy 21q: a new benign chromosomal variant. *Eur J Med Genet* 2007; **50**: 54–59.
13 Giorda R, Cerritello A, Bonaglia MC *et al*: Selective disruption of muscle and brain-specific *BPAG1* isoforms in a girl with a 6;15 translocation, cognitive and motor delay, and tracheo-oesophageal atresia. *J Med Genet* 2004; **41**: e71.
14 Stoll C, Roth MP: Segregation of a 22 ring chromosome in three generations. *Hum Genet* 1983; **63**: 294–296.
15 Tommerup N, Warburg M, Gieselmann V, Hansen BR, Koch J, Petersen GB: Ring chromosome 22 and neurofibromatosis. *Clin Genet* 1992; **42**: 171–177.
16 Woods CG, Bankier A, Curry J *et al*: Asymmetry and skin pigmentary anomalies in chromosome mosaicism. *J Med Genet* 1994; **31**: 694–701.
17 Chen CP, Chern SR, Chang TY *et al*: Prenatal diagnosis of mosaic ring chromosome 22 associated with cardiovascular abnormalities and intrauterine growth restriction. *Prenat Diagn* 2003; **23**: 40–43.
18 Yong YP, Knight LA, Yong MH, Lam S, Ho LY: Partial monosomy for chromosome 22 in a girl with mental retardation. *Singapore Med J* 1997; **38**: 85–86.
19 Riegel M, Baumer A, Wisser J, Acherman J, Schinzel A: Prenatal diagnosis of mosaicism for a del(22)(q13). *Prenat Diagn* 2000; **20**: 76–79.
20 Schinzel AA, Basaran S, Bernasconi F, Karaman B, Yuksel-Apak M, Robinson WP: Maternal uniparental disomy 22 has no impact on the phenotype. *Am J Hum Genet* 1994; **54**: 21–24.
21 Henry I, Puech A, Riesewijk A *et al*: Somatic mosaicism for partial paternal isodisomy in Wiedemann-Beckwith syndrome: a post-fertilization event. *Eur J Hum Genet* 1993; **1**: 19–29.
22 Meselson M, Weigle JJ: Chromosome breakage accompanying genetic recombination in bacteriophage. *Proc Natl Acad Sci USA* 1961; **47**: 857–868.
23 Kraus E, Leung WY, Haber JE: Break-induced replication: a review and an example in budding yeast. *Proc Natl Acad Sci USA* 2001; **98**: 8255–8262.

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