Molecular characterization of a mosaicism with a complex chromosome rearrangement: evidence for coincident chromosome healing by telomere capture and neo-telomere formation

Elyes Chabchoub, Laura Rodríguez, Enrique Galán, Elena Mansilla, María Luisa Martínez-Fernández, María Luisa Martínez-Frias, Jean-Pierre Fryns and Joris R Vermeesch

J. Med. Genet. published online 15 Dec 2006;
doi:10.1136/jmg.2006.045476

Updated information and services can be found at:
http://jmg.bmj.com/cgi/content/abstract/jmg.2006.045476v1

Rapid responses You can respond to this article at:
http://jmg.bmj.com/cgi/eletter-submit/jmg.2006.045476v1

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Notes

Online First contains unedited articles in manuscript form that have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Online First articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Online First articles must include the digital object identifier (DOIs) and date of initial publication.

To order reprints of this article go to:
http://www.bmjjournals.com/cgi/reprintform

To subscribe to Journal of Medical Genetics go to:
http://www.bmjjournals.com/subscriptions/
Molecular characterization of a mosaicism with a complex chromosome rearrangement: evidence for coincident chromosome healing by telomere capture and neo-telomere formation

Elyes Chabchoub, Laura Rodríguez, Enrique Galán, Elena Mansilla, Maria Luisa Martínez-Fernandez, Maria Luisa Martínez-Frías, Jean-Pierre Fryns and Joris Robert Vermeesch

E Chabchoub, J-P Fryns, JR Vermeesch, Center for Human Genetics, University Hospital Gasthuisberg, Leuven; Belgium
L Rodríguez, E Mansilla, ML Martínez-Fernandez, ML Martínez-Frías, Estudio Colaborativo Español de Malformaciones Congénitas (ECEMC) del Centro de Investigación sobre Anomalías Congénitas (CIAC), Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Madrid, Spain
E Galán, Servicio de Pediatría. Hospital Materno Infantil de la seguridad Social de Badajoz, Badajoz, Spain
ML Martínez-Frías, Departamento de Farmacología, Facultad de Medicina, Universidad Complutense. Madrid, Spain

Correspondence to: J.R. Vermeesch, Center for Human Genetics, Herestraat 49, 3000 Leuven, Belgium; Joris.Vermeesch@uz.kuleuven.ac.be; Phone: +32 16 345941; Fax: +32 16 346060

KEY WORDS: Telomere capture, Neo-telomere formation, Complex chromosome rearrangements, Mosaicism, Array CGH

Word count: Abstract: 210 words
Main text: 3202 words
ABSTRACT

Background: Broken chromosomes must acquire new telomeric “caps” to be structurally stable. Chromosome healing can be mediated either by telomerase through *neo*‐telomere synthesis or by telomere capture.

Aim: To unravel the mechanism(s) generating complex chromosomal mosaicism and healing broken chromosomes.

Methods: G banding, array comparative genomic hybridization (aCGH), fluorescent *in‐situ* hybridization (FISH) and short tandem repeat analysis (STR) was performed on a girl presenting with mental retardation, facial dysmorphism, urogenital malformations and limb anomalies carrying a complex chromosomal mosaicism.

Results & discussion: The karyotype showed a *de novo* chromosome rearrangement with two cell lines: one cell line with a deletion 9pter and one cell line carrying an inverted duplication 9p and a non‐reciprocal translocation 5pter fragment. aCGH, FISH and STR analysis enabled the deduction of the most likely sequence of events generating this complex mosaic. During embryogenesis, a double‐strand break occurred on the paternal chromosome 9. Following mitotic separation of both broken sister chromatids, one acquired a telomere *via* neo‐telomere formation, while the other generated a dicentric chromosome which underwent breakage during anaphase, giving rise to the del inv dup(9) that was subsequently healed by chromosome 5 telomere capture.

Conclusion: Broken chromosomes can coincidently be rescued by both telomere capture and *neo*‐telomere synthesis.
INTRODUCTION
Telomeres are specialized nucleoproteic complexes localized at the physical ends of linear eukaryotic chromosomes that maintain their stability and integrity.[1] Telomere loss causes chromosome instability (through breakage or improper telomere maintenance) resulting in several types of chromosome rearrangements, including terminal deletions, inverted duplications, DNA amplification, duplicative and non-reciprocal translocations, and dicentric chromosomes, all of which have been associated with human diseases, cell senescence, and/or apoptotic cell death. Such chromosome aberrations can be prevented or terminated by the addition of telomeric repeats to the end of the broken chromosome.[2] Telomerase, a specialized reverse transcriptase-like enzyme, can stabilise chromosomal broken ends by the addition of telomeric sequences directly on to non-telomeric DNA. Telomerase is activated in cancer cells and in germ line cells and is still active in early stages of embryogenesis.[3] Broken chromosomes can acquire new telomeres by “telomere capture”, a process first described by Meltzer et al.[4] in cancer cells, transformed fibroblasts and lymphoblastoid cell lines. This process involves the addition of telomeres from normal chromosomes at the site of double-strand breaks (DSBs) to stabilize broken chromosomes by non reciprocal translocation.[4] Schematically, the broken end of a chromosome invades a region of homology and initiates replication, thereby duplicating the end of that chromosome.[5] Particular chromosomal anomalies, such as mosaicism, can help to elucidate some aspects of chromosome healing and increase further our understanding of the mechanism of genomic disorders. Constitutional chromosomal mosaicism with two cell lines carrying two different rearranged sets of chromosomes is an extremely rare condition that is generally the result of a post-fertilisation mitotic error.[6-7] Polymorphic markers analysis showed that, in addition, such mosaics may originate during parental meiosis.[7-14] In this paper we describe a girl with a mosaic del(9)/der(9)t(5;9)inv dup(9) initially diagnosed by conventional high resolution G-banded chromosomes and characterized by array-comparative genomic hybridization (a CGH) and fluorescence in-situ hybridization (FISH). The most straightforward explanation for our findings would be an early post-zygotic error followed by independent chromosome healing of both sister chromatids by neo-telomere formation and telomere capture.

PATIENTS AND METHODS
Clinical report (fig 1A-B)
The patient was a newborn Spanish girl product of the first pregnancy of healthy and non-consanguineous parents. The father and the mother were 19 and 16 years old respectively. The pregnancy was complicated by oligohydramnios. The delivery was at term and eutocic. The birth weight was 2.700 g and the clinical examination showed trigonocephaly and bilateral club feet.

When she was 8 months old, clinical examination showed other dysmorphic features with upslanting palpebral fissures, depressed and broad nasal root, asymmetric implantation of the ears, bifid uvula, normal palate, plagiocephaly and prominent metopic suture. Cardiac examination revealed a heart murmur but no structural cardiac anomalies. Labia majora were hypoplastic. Cranial X-ray was normal. Abdominal ultrasound showed a hypoplastic and ectopic right kidney and retrograde urography a grade III vesicoureteral reflux on the left kidney and a grade I vesicoureteral reflux on the right. These refluxes had disappeared at the age of 3 years.

The girl was able to sit unsupported at 9 months old and walked alone at 18 months. Physical examination at 6 years old showed mild synophrys, hypoplastic alae nasi, long and smooth philtrum, thin upper lip, small and dysmorphic ears, pectus excavatum and camptodactily of the fifth fingers. She had a moderate psychomotor delay.
At 10 years, she was not able to read. She had dental caries and an angioma appeared on the internal side of the left lower limb. Menarche was at normal age (13 years). At present, she is 14 years old. Height is 146 cm (P3-P10), weight 55 kg (P75) and head circumference (OFC) 52.5 cm (P3-P10).

**Cytogenetic analysis**  
High resolution G banded chromosomes were prepared from peripheral blood lymphocytes according to standard procedures.

**Array-CGH analysis**  
DNA of the parents and the child was extracted from peripheral white blood cells according to standard procedures. Array CGH was performed as previously described.[15-16] Briefly, for total genome coverage array CGH, arrays were constructed using a 1 Mb clone set which contains 3587 BAC and PAC clones spotted in double. Test and reference genomic DNAs were labelled by a random prime labelling system (Bioprime array CGH, Invitrogen) using Cy3- and Cy5-labeled dCTPs (Amersham Biosciences). The results presented are a combination of two hybridisations in which patient and a parent (mother and father’s DNA labelling) was dye swapped in a loop design.

A chromosome 9 full tiling path array-chip was constructed using 560 BAC and PAC clones from the 32K BAC clone library (CHORI BACPAC Resources, http://bacpac.chori.org/genomicRearrays.php) and 595 BAC clones from the 1 Mb clone set mapped to various human chromosomes as internal controls. Experiments have been proceeded and data analysed as for the 1 Mb array-CGH.

**FISH analysis**  
FISH was performed on metaphase and nuclei spreads according to standard procedures with probes labelled either by biotine-16-dUTP (Boehringer Mannheim®)[14] or by DOP-PCR direct labelling with SpectrumOrange™-dUTP (Vysis®, Abbott Molecular Inc.).[15] For chromosome 5, the commercial probes Cytocell® Aquarius LPU008 specific for the Cri-du-Chat chromosome region (CDCCR) and Cri-du-Chat Syndrome LSI® D5S23, D5S71 SpectrumGreen™ (Vysis®, Abbott Molecular Inc.) were used according to the respective manufacturer’s protocols. The FITC-(C3TA2)3 peptide nucleic acid (PNA) telomeric probe was used for FISH experiments to detect telomeric repeats in cells spreads using the regular FISH protocol.

**DNA polymorphism analysis**  
A set of microsatellites polymorphic markers (CA)n repeats spaced along the chromosomes 5 and 9 was selected and amplified by polymerase chain reaction (PCR) in 35 cycles using fluorescently labelled primers (a 6-FAM 5’label on the forward primers). Primer sequences and loci information are available from the Genome Database (GDB) (http://www.gdb.org/gdb/) and the NCBI database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists). The amplicons were sized by capillary electrophoresis on an ABI PRISM® 3100 Genetic Analyzer. Size of the alleles and area of the peaks were calculated with GeneScan 3.1® and Genotyper 3.7® softwares (Applied Biosystems). In order to assess whether duplication had occurred at any given locus, a quantitative analysis was performed. The area of each allelic peak (a measure of the amount of amplified material) and the ratio between the areas of the shorter and longer allele were calculated.

**Bioinformatics breakpoints sequences analysis**
Pairwise BLAST searches using the genomic sequences (based on NCBI build 36.1) of regions spanning breakpoints on 5p13.3, 9p22.1 and 9p13.3 were performed using NCBI's Blast 2 Sequences online platform (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi).[17] In order to identify large stretches (>1 kb) of high sequence identity (>90%), such as that found in low-copy repeats (LCRs), for each pairwise BLAST analysis the search parameters were adjusted as follows: the Expect threshold was lowered to 5 to increase the stringency of the search; the Word Size was increased to a value of 100 to search for longer stretches of homology; and the Filter option was selected to mask out low-complexity and repetitive DNA sequences. Sequence homologies and search for LCRs were performed on the above mentioned sequences using PipMaker online software (http://pipmaker.bx.psu.edu/cgi-bin/pipmaker?basic). Possible homology regions detected by PipMaker were checked simultaneously by BLAT platform using the UCSC Human BLAT Search tool (http://genome.ucsc.edu/cgi-bin/hgBlat) and BLAST using the NCBI BLASTn software (http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

Cytogenetic analysis
High resolution G banding showed the presence of two cell lines in the peripheral blood lymphocytes: 46,XX,del(9)(p22.1)[21]/46,XX,der(9)t(5;9)(p13.3;p22.1)[18] indicating the presence of monosomy 9p22.1 in all blood cells and trisomy 5p13.3 in half of blood cells (fig 2A). The chromosomes of the parents were normal.

Array-CGH analysis
To characterise the rearranged regions, array CGH was performed on DNA from the patient and his parents (fig 3A-C). The hybridisation efficiency of the experiment was 94.68% with a standard deviation (S.D.) of 0.088. The 1 Mb resolution genome wide array CGH showed an approximately 18.5 Mb terminal deletion of chromosome 9p with interstitial duplication of chromosome 9p of approximately 16.5 Mb and a terminal duplication of chromosome 5p of about 28.8 Mb, extending to the region between the clones RP11-46C20 and RP11-37M16 (fig 3B). The chromosome 9 tiling path array enabled finemapping of the breakpoints on the chromosome 9 (fig 3C). The deleted region was shown to span in-between BACs RP11-269B5 and RP11-296P7. This represents also the distal breakpoint (the telomeric one) for the interstitial duplicated region, while the proximal one was flanked by BAC RP11-284F1 and BAC RP11-182L18. The average log2 of the intensity ratio values of the abnormal clones duplicated on chromosomes 5p and 9p were respectively 0.23 and 0.25. Since the theoretical intensity ratio of a duplication is log2(3/2)=0.58 (3 copies odds 2 copies in a normal situation), the estimated degree of mosaicism would be 0.23/0.58 to 0.25/0.58 or 40% to 43%. These abnormalities were shown to be de novo, since arrays of the parents were normal (data not shown).

FISH analysis: Organisation of rearranged chromosomes within both of the cell lines
In order to confirm the array CGH results and to determine how the deletion and duplications are organized in both cell lines, a series of BAC probes was hybridised on metaphase spreads of the patient lymphocytes and investigated by FISH (fig 2B-E). In 96 out of 100 nuclei and 16 metaphases analysed, probes RP11-48M17 and RP11-503K16 spanning the deleted region on the 1Mb array showed, each, only one signal, i.e. all the cells had the del(9p) chromosome (fig 2B). As expected, probes RP11-513M16 (SpectrumOrange™ labelled) and RP11-48L13 (biotine labelled) localized at the duplicated region showed three signals in half of the studied nuclei (45 out of 100) (which is in accord with arrays findings). On metaphases, we could see a green-red-green order of the probes, suggesting an inverted duplication as shown in fig 2C.
Besides, all the inv dup(9p) had the terminal deletion. To study the mosaicism for the duplicated 5p, metaphase spreads from the patient were hybridized with BAC RP11-513M16 together with the commercial probe for the Cri-du-Chat Syndrome (CDCCR) LSI® D5S23, D5S71 SpectrumGreen™ (fig 2D). Both chromosomes 5 where hybridized in all analysed metaphases whereas in half of them, a red signal corresponding to the CDCCR specific probe hybridized on 9p, telomeric to the BAC RP11-513M16, demonstrating a non reciprocal translocation of the 5p on top of the 9p in half of the analysed cells (as calculated with the mean of arrays intensity ratios). To characterize the cell line affected by this non reciprocal translocation, we carried out a FISH with the commercial probe Cytocell® Aquarius LPU008 (specific for the CDCCR in 5p) and both of the BACs RP11-513M16 (SpectrumOrange™ labelled) and RP11-48L13 (biotine labelled) (fig 2E). As expected, we demonstrated that the duplicated 5p was located telomeric to all the simultaneously del dup(9p) chromosome. FISH confirmed that the mosaic duplicated region of chromosome 5p extended to the BAC clone RP11-53L13 on 5p13.3 (data not shown). In addition, peptide nucleic acid (PNA) telomeric probe hybridized to all chromosomes ends (data not shown) demonstrating the presence of telomeric sequences capping the ends of the broken chromosomes.

**DNA polymorphism analysis: Origin of rearrangements**

To investigate the origin of the mosaicism and confirm the FISH analysis, polymorphic marker analysis was performed on DNA extracted from peripheral blood lymphocytes of the patient and his parents (table 1).
Table 1. Results of the polymorphic markers analysis

<table>
<thead>
<tr>
<th>Marker</th>
<th>Position (ISCN 2005)</th>
<th>Location (Mb-NCBI)</th>
<th>{Genotypes / Allele values}</th>
<th>Parental Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Father</td>
<td>Propositus</td>
</tr>
<tr>
<td>D5S1981</td>
<td>5p15.33</td>
<td>1.2</td>
<td>257</td>
<td>257/263</td>
</tr>
<tr>
<td>D5S406</td>
<td>5p15.32</td>
<td>5</td>
<td>173</td>
<td>173</td>
</tr>
<tr>
<td>D5S630</td>
<td>5p15.31</td>
<td>9.6</td>
<td>238</td>
<td>238/244</td>
</tr>
<tr>
<td>D5S1991</td>
<td>5p15.2</td>
<td>15</td>
<td>223/229</td>
<td>223/225</td>
</tr>
<tr>
<td>D5S618</td>
<td>5q14.3</td>
<td>89</td>
<td>168/172</td>
<td>172</td>
</tr>
<tr>
<td>D5S671</td>
<td>5q34</td>
<td>163</td>
<td>201</td>
<td>201</td>
</tr>
<tr>
<td>D5S2073</td>
<td>5q35</td>
<td>195</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>D9S129</td>
<td>9p24.3</td>
<td>1.85</td>
<td>131</td>
<td>129</td>
</tr>
<tr>
<td>D9S178</td>
<td>9p24.2</td>
<td>4</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>D9S269</td>
<td>9p23</td>
<td>11</td>
<td>168/174</td>
<td>168</td>
</tr>
<tr>
<td>D9S285</td>
<td>9p22.3</td>
<td>15</td>
<td>118/120</td>
<td>122</td>
</tr>
<tr>
<td>D9S1846</td>
<td>9p21.3</td>
<td>21.6</td>
<td>182/188</td>
<td>182/186</td>
</tr>
<tr>
<td>D9S974</td>
<td>9p21.3</td>
<td>21.9</td>
<td>208/210</td>
<td>204/210</td>
</tr>
<tr>
<td>D9S942</td>
<td>9p21.3</td>
<td>21.9</td>
<td>112/118</td>
<td>102/112</td>
</tr>
<tr>
<td>D9S1748</td>
<td>9p21.3</td>
<td>21.9</td>
<td>106/116</td>
<td>110/116</td>
</tr>
<tr>
<td>D9S171</td>
<td>9p21.3</td>
<td>24.5</td>
<td>163/169</td>
<td>155/169</td>
</tr>
<tr>
<td>D9S165</td>
<td>9p13.3</td>
<td>33</td>
<td>210/212</td>
<td>212/216</td>
</tr>
<tr>
<td>D9S970</td>
<td>9p13.1</td>
<td>39</td>
<td>137/141</td>
<td>137/141</td>
</tr>
<tr>
<td>D9S197</td>
<td>9q22</td>
<td>66</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>D9S127</td>
<td>9q31</td>
<td>77</td>
<td>152/154</td>
<td>154/156</td>
</tr>
<tr>
<td>D9S1199</td>
<td>9q34</td>
<td>134,8</td>
<td>89/93</td>
<td>93/99</td>
</tr>
<tr>
<td>D9S1793</td>
<td>9q34</td>
<td>135,4</td>
<td>175/185</td>
<td>177/185</td>
</tr>
<tr>
<td>D9S66</td>
<td>9q34</td>
<td>135,7</td>
<td>119/129</td>
<td>119/129</td>
</tr>
<tr>
<td>D9S1818</td>
<td>9q34</td>
<td>136,3</td>
<td>154</td>
<td>148/154</td>
</tr>
<tr>
<td>D9S312</td>
<td>9q34</td>
<td>137,1</td>
<td>121</td>
<td>117/121</td>
</tr>
<tr>
<td>D9S1826</td>
<td>9q34</td>
<td>137,6</td>
<td>129/133</td>
<td>131/133</td>
</tr>
<tr>
<td>D9S158</td>
<td>9q34</td>
<td>138,3</td>
<td>216/218</td>
<td>216/218</td>
</tr>
<tr>
<td>D9S1838</td>
<td>9q34</td>
<td>139,8</td>
<td>167</td>
<td>161/167</td>
</tr>
</tbody>
</table>

* pat dup = paternal duplication (interpretation based on the dosage analysis)
** pat del = paternal deletion
*** NI = Non informative

Seven polymorphic markers along chromosome 5 and twenty along chromosome 9 were analysed. Three markers along chromosome 5 and ten others along chromosome 9 were outside the rearranged region. A single paternal and maternal allele could be seen and confirmed that one chromosome is derived from the father and the other from the mother in both cell lines (fig 4, D5S618 and D9S1793).

Markers D9S129 and D9S285 show that only a single maternal allele was detected in 9pter (fig 4). The del(9p) is thus from paternal origin.

Markers derived from the duplicated regions on chromosomes 5 and 9 showed a single allele from the mother and a single one from the father. However, the peak areas were not in a one to one ratio but in a 1.62±0.17 to one ratio for father’s allele to mother’s one (fig 4, D5S1981 and D9S1846). This dosage analysis indicates the presence of two paternal copies in half of the blood leucocytes and is consistent with a single chromosome from the mother and a duplication in the chromosome derived from the father (where the ratio would be a $3/2$ to 1).
The duplication would be an intrachromosomal duplication since only a single allele from the father could be detected.

This confirms the conventional and molecular cytogenetic findings that BACs containing these polymorphic markers are duplicated on the inv dup(9p) and on the dup(5p) and present in a single copy on the del(9p).

The final karyotype could be written as follows:

```plaintext
46,XX,del(9)(p22.1).arr cgh 9pter-22.1(RP11-269B5)x1.ish del(9)(p22.1)
(48M17-,503K6-,513M16+)dn[55]/ 46,XX,der(9)t(5;9)(p13.3;p22.1).arr cgh
5pter13.3(RP11-415K6->RP11-53L13)x3, 9pter22.1(GS1-77L23->RP11-269B5)x1,
9p22.1p13.3(RP11-296P7->RP11-269B5)x1, 9p22.1p13.3(RP11-296P7->RP11-284F1)x3.ish
der(9)t(5;9)(p13.3;p22.1)del(9)(p22.1)dup(9)(p13.3->p22.1->qter)
(53L13++,15G6++;48M17-,503K6-,513M16++,48L13++)dn[45].
```

**Bioinformatics breakpoints sequences analysis**

Sequence analysis with PipMaker did unravel the presence of high level of homology (>95%) sequences of a nearly 6 Kb in the breakpoint regions on chromosomes 5p13.3, 9p22.1 and 9p13.3. This was confirmed in-silico by pairwise blast using BLAST2 search (data not shown). The sequences were identified in Ensembl database v38 (http://www.ensembl.org/Homo_sapiens/) to LINE repeats known as L1PA3 with its homologous L1PA2 on chromosome 5 and L1PA7 on chromosome 9.

**DISCUSSION**

**Mechanism**

Broken chromosomes can be healed by two general pathways: either by telomerase through neo-telomere synthesis or by telomere capture. Here we provide the evidence for an independent involvement of neo-telomere synthesis and telomere capture in the chromosome rescue process through the description of a patient presenting a mosaic del(9)/der(9)t(5;9)inv dup(9). The simplest model to describe the sequence of events generating this mosaicism is depicted in figure 5. We assume that in the present patient, a DSB in the paternal chromosome 9p initiated the genomic disorder early during embryogenesis. The deletion breakpoint in the del(9) and the start of the duplication in the inv dup(9) coincide. Hence, the del(9) is not the reciprocal product of the inv dup(9). Therefore, we hypothesise that during S-phase the broken chromatid replicated and the cell underwent mitosis. In one daughter-cell, telomerase catalysed the addition of telomeric sequences on to the broken del(9). This mechanism is known as neo-telomere formation. In the other daughter-cell, a fusion of the two sister chromatids lead to a dicentric chromosome 9. During anaphase of the next mitosis, both chromatids of that dicentric chromosome 9 would be pulled to the opposite poles of the cell, leading to their disruption in p13.3 resulting in an inv dup(9p) in one daughter cell and del(9p) in the other. A telomere capture by non reciprocal translocation of the chromosome 5p stabilized the inv dup(9). The chromosome 5 subtelomere capture event might have occurred by a breakage induced recombination event, thus leaving the original chromosome 5 intact or might have generated another cell line with a del(5) which we did not detect in our patient.[5,18-19] Another possibility exists that following the generation and stabilisation of the inv dup(9) this chromosome was broken at the same locus as the initial breakage event. However, it seems unlikely that a breakage would occur independently twice at the same 100 kb interval.

While this sequel may appear a unique chain of events, recently two similar cases have been described. Kulikowski et al.[10] reported a girl with an equal ratio mosaic of two cell lines presenting a monosomy 9p23 in all cells and a trisomy 1q41 in half of the cells. No further investigations to elucidate the mechanisms originating the abnormal chromosomes in
both cell lines were performed by the authors. Reddy and Yang[20] describe the cytogenetic analysis of a patient with a mosaic del(1)/der(1)t(1p;9p). They also invoked independent telomere stabilization by telomerase and telomere capture of the sister chromatids. In this case, no inverted duplication was detected. The presence of such a duplication may have been overlooked or the 9p telomere capture occurred without the formation of a dicentric chromosome.

**A post-zygotic mosaic**

In a recent review, Pramparo et al.[11] suggested that constitutional chromosomal mosaicism with two cell lines carrying two different rearranged chromosomes, might arise following a meiotic error. In this case, both sister chromatids have separated either during the second meiotic cell division (MII) or during post-zygotic mitosis. If the breakage would have occurred during MII, it can be expected that during MII bivalent 9 would have undergone canonic recombination involving the q arms and as a consequence, we would expect two paternal alleles on 9q, one in each of both cell lines. Telomeric polymorphic markers on chromosome 9q have shown only one paternal and one maternal allele. Therefore, in this patient, the mosaicism originated most likely during embryogenesis.

**LINEs mediating the rearrangement?**

Breakpoints clustering in low copy repeats (LCRs) are usually responsible for rearrangements by the mechanism of Non-Allelic Homologous Recombination (NAHR), whereas rearrangements with scattered breakpoints, other mechanisms such as Non-Homologous End Joining (NHEJ) have been observed.[21] Rearrangements of chromosome 9 do not show site-specific breakpoints.[22-26] Therefore, NHEJ seems to be the most likely mechanism of rearrangements for chromosome 9p.[27] *In-silico* sequence analysis of chromosomes 5 and 9 showed the existence in the breakpoint region 5p13.3 of L1PA2, a LINE sequence with homology with L1PA3 in the breakpoint regions in 9p13.3 and in 9p22.1 and with L1PA7 in 9p22.1. L1PA3 with its homologous L1PA2 are duplicated in four copies in 5p13.3. This LINE-1 element could mediate the NHEJ generating the non-reciprocal translocation of chromosome 5p healing the inv dup(9p).

**Clinical Considerations:**

This patient shows the major clinical manifestations described in the 9p deletion syndrome that comprises mental retardation, hypotonia, trigonocephaly, upslanting palpebral fissures, flat nasal bridge with anteverted nares, long filtrum and small malformed ears. Christ et al.[22] performed karyotype-phenotype correlations in patients with 9p- mapping its critical region to a 4-6 Mb in 9p22-23.

However our patient has a mosaic partial duplication of 5p and 9p which may modulate the chromosome 9p partial deletion clinical presentation. Partial dup(9p) in tandem, seems to give a different clinical presentation from the inv dup(9p). Fryns et al.[28] described a girl with tandem dup(9)(p13;p22) and trisomy 9p phenotype presenting with a mild mental retardation, downslanting palpebral fissures, hypotonia without prominent metopic ridge, whereas in a 20 month-old girl with inv dup(9)(p22;p13), Teebi et al.[25] found a psychomotor and developmental delay with hypotonia, prominent metopic ridge and upslanting palpebral fissures.

Partial trisomy 5p is a very rare event, first described in 1964 by Lejeune. Most partial trisomies 5p are the consequence of an unbalanced translocation with another autosome.[27] Partial trisomies involving 5p14-pter do not show a particular dysmorphism, while those including 5p13 or the complete short arm have more severe multiple congenital anomalies, mental retardation, and growth failure.[27] So far, Liberfarb et al.[29] reported at least four
members having 5p+/9p- in a large translocation carrier family with features of
dup(5)(pter→p13) and del(9)(pter→p22). All died in early childhood between 4 and 27
months from recurrent infections. At least two had prominent forehead, flat nasal bridge,
arachnodactyly, bilateral clubfeet, diaphragmatic and umbilical hernias, intestinal and kidney
malformations. One of them had marked psychomotor retardation and brain malformations.
The severe presentation could be due to the association of the 5p+ and 9p-. Our patient has the
same chromosomal abnormalities, but she has milder phenotype. This could be explained by
the mosaic 5p+ and/or the mosaic 9p+ which may balance the defect on chromosome 9.

In conclusion, by using molecular techniques we gave evidence for involvement of
both neo-telomere formation and telomere capture in chromosome healing of constitutional
chromosome rearrangements.

ACKNOWLEDGEMENTS
We would like to thank the patient and her parents for their precious collaboration. Elyes
Chabchoub was supported by the Ministry of Higher Education from Tunisia (Scholarship
2005-032/001). The authors wish to thank the MicroArray Facility, Flanders Interuniversity
Institute for Biotechnology (VIB) for their help in the spotting of the arrays and the Mapping
Core and Map Finishing groups of the Wellcome Trust Sanger Institute for the initial clone
supply and verification. This work was made possible by grants G.0200.03 from the FWO,
OT/O2/40, GOA/2006/12 and Centre of Excellence SymBioSys (Research Council
K.U.Leuven EF/05/007), Catholic University of Leuven, and grant (PI020028) from the
Fondo de Investigaciones Sanitarias (FIS), Instituto de Salud Carlos III, Ministerio de Sanidad
y Consumo, Spain.
LEGENDS

Figure 1 A-B Clinical pictures of the patient.

Figure 2 Cytogenetic data. (A) Partial high resolution G banded karyotype showing the normal chromosome 9 and the del(9) on the left and a normal chromosome 9 and the der(9) on the right. Ideograms of the normal and the derivative chromosomes 9 are shown. (B-E) FISH data with a schematic representation of the probes loci on the ideograms as expected. (B-A del(9p) cell: The picture shows the probes tested for the 9p terminal deletion, with only one signal for both of the probes on a metaphase and a nuclei (white arrow). (C) FISH shows the inv dup(9p). (D-E) FISH analysis displaying the organisation of the rearranged chromosomes within the der(9p) cell line with a schematic representation of the loci of the probes on ideograms of the chromosomes 5 and der(9). The black line represents the region of the chromosome that belongs to 9, the red line the one belonging to chromosome 5p and the green line the inv dup segment of chromosome 9. The thick arrowheads indicate chromosome 9 and the thin ones indicate chromosome 5. (D) This plate shows the non reciprocal translocation of 5p on chromosome 9. (E) FISH using Cri-du-Chat Syndrome LSI® D5S23, D5S71 SpectrumGreen™ demonstrating that the dup(5p) is situated telomeric to the der(9p). The ideogram of the normal chromosome 9 with probes assigned on it, is the same as the one on the left of plate C.

Figure 3 Array CGH data. (A) Result of the 1Mb array CGH analysis of the patient. The y-axis represents the log2 of the intensity ratios of the combined dye swap experiments of the patient/parental DNA within the loop design. In the x-axis clones are ordered from the short-arm telomere to the long-arm telomere and chromosomes are ordered from 1 to 22. The green lines indicate the thresholds (4x S.D.) for clone deletion (-0.36) and duplication (+0.36). The aberrant clones are encircled in red. (B) Partial 1Mb array-CGH data from chromosome 5 of the child displaying log2 ratio plot with the mosaic duplicated region of 5p delineated by the green upward arrowheads. In the X axis the relative distance of the BAC clones from the 5p telomere is indicated in Mb with the ideogram. (C) A full tiling path 32k array CGH of chromosome 9 of the patient displaying the deletion (red downward arrowheads) and the mosaic duplication (green upward arrowheads) and enabling the finemapping of breakpoints.

Figure 4 Polymorphism analyses of D5S1981, D5S618, D9S129, D9S285, D9S1846 and D9S1793 on DNA extracted from blood of the patient (P), the mother (M) and the father (P). The size of the allele can be estimated from the scale generated by the Genotyper® software.

Figure 5 Schematic representation of the proposed mechanism of origin of the rearrangements during early embryonic development (refer to text for detailed description). Only chromosomes 9 are shown. Dotted lines in F indicate spindles attaching to the centromeres. The flashing red arrow represents the DSBs sites. The blue dots indicate the new telomeres. The green dots indicate the inv dup(9p) fragment. The chromosome 5pter derived fragment is represented in red. Stages: A-B: DSBs event in the embryo. C: Metaphase. D: Telophase. E: Metaphase 1 neo-telomere synthesis event, 2 fusion of the broken sister chromatids. F: Anaphase-second breakage. G: Telophase, generation of two cell lines. H: Telomere capture event.
REFERENCES

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in JMG and any other BMJPGL products to exploit all subsidiary rights, as set out in our licence (http://jmg.bmjjournals.com/ifora/licence.pdf)"
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5