Mosaicism for combined tetrasomy of chromosomes 8 and 18 in a dysmorphic child: A result of failed tetraploidy correction?

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Background: Mosaic whole-chromosome tetrasomy has not previously been described as a cause of fetal malformations.

Case presentation: In a markedly dysmorphic child with heart malformations and developmental delay, CGH analysis of newborn blood DNA suggested a 50% dose increase of chromosomes 8 and 18, despite a normal standard karyotype investigation. Subsequent FISH analysis revealed leukocytes with four chromosomes 8 and four chromosomes 18. The child’s phenotype had resemblance to both mosaic trisomy 8 and mosaic trisomy 18. The double tetrasomy was caused by mitotic malsegregation of all four chromatids of both chromosome pairs. A possible origin of such an error is incomplete correction of a tetraploid state resulting from failed cytokinesis or mitotic slippage during early embryonic development.

Conclusion: This unique case suggests that embryonic cells may have a mechanism for tetraploidy correction that involves mitotic pairing of homologous chromosomes.

Background

Unlike meiotic non-disjunctions, mitotic non-disjunctions are rarely observed in humans with the exception of mosaicism for trisomy 8, 9 or 20 [1,2]. In some cases mosaic trisomy of more than one chromosome have been seen [3]. Such mosaic variegated aneuploidy is due to mitotic errors, often associated with premature centromere division [4]. In contrast to mosaic trisomies, the finding of mosaic whole-chromosome tetrasomy is without precedence. Here we present such a patient; a dysmorphic newborn child with mosaicism of leukocytes containing 50 chromosomes due to tetrasomy of chromosomes 8 and 18. This unique clinical case may have relevance concerning the origin of aneuploidy in cancer [5-8] because it indirectly suggests that there might be a mechanism for tetraploidy correction during fetal development that involves mitotic pairing of homologous chromosomes.

Case presentation

A baby girl was delivered by cesarean section in week 36 due to maternal hypertension with mild preeclampsia, birth weight 2910 g, length 47 cm. Polyhydramnios was detected at the end of the pregnancy. She had persistent ductus arteriosus (PDA), a small muscular-type ventricle septal defect (VSD) and coarctation of the aorta. The coarctatio aortae was resected at age 6 weeks, and at the same time the PDA was ligated. She has always been short stuated: At age 4 months her length was 57 cm (1 cm below 2.5th centile), at age 2 1/2 years 81 cm (4 cm below...
2.5th centile). Head circumferences were about 1 cm below the 2.5th centile, e.g. 46 cm at age 2 1/2 years. Major feeding difficulties necessitated gastrostomy at age 4 months. At current age (2 years and 10 months) she still has no interest for food and vomits easily, but feeding her through the enteral feeding tube (Mic-Key®) keeps her weight within normal range. On barium-contrast X-ray examination of the esophagus, peristalsis appeared normal without signs of gastro-esophageal reflux. A 24-hour esophageal pH-measurement also gave no indications of reflux. There has been clear psychomotor delay. She started to walk without support at age 2 years and has delayed language development, e.g. at age 2 1/2 years she spoke only 8–10 words but managed quite well by sign language. On neurological examination mirror movements of her hands were found. She also has hearing loss, already suspected before age 2 months and confirmed by brain stem audiometry at age 4 months. On CT-examination of the temporal bone, atretic auditory canals and no middle ear cavities were found. There is marked facial dysmorphism with high frontal hairline, low-set and posteriorly rotated small ears with crumpled helices, inverted epicanthus, short and down-slanting palpebral fissures, no visible eyelashes on lower eyelids, broad nasal root, thin upper lip, small chin and a short neck (Figure 1). A transverse palmar crease was found in the right hand, and on the inside of the left thigh brownish longitudinal streaks were seen. The right foot was deformed with the 1st toe bent in under the 2nd toe (Figure 1). On ophthalmological examination in narcosis, the optic papillae were grayish, and there were some granulations of the retinae in the macular areas. At present, she is an active girl that likes to play. Her only medication is for asthma.

**Results and Discussion**

Because the girl was dysmorphic with major feeding difficulties, blood samples were drawn two days after birth for chromosome investigations; routine G-banding and chromosome-based high-resolution comparative genomic hybridization (HR-CGH). The G-banded karyotype, based on screening of ten metaphases from a phytohemagglutinin-stimulated 3-day blood lymphocyte cultures, was normal. Surprisingly, the HR-CGH result that came a few weeks later suggested a combination of non-mosaic trisomy 8 and trisomy 18 (Figure 2, panel A). An identical finding was subsequently done on a 3500 BAC-clone array-CGH platform made by the Norwegian Microarray Consortium (for details, see [9]), the ratio still indicating a 50% increase in DNA amount corresponding to chromosomes 8 and 18 (Figure 2, panel B). To investigate if uniparental disomy of other chromosomes might be present, or if abnormal copy number variants below the resolution limit of the 3500 BAC-clonet array might be found, we have recently examined DNA from the child's

**Figure 1**
The child with double tetrasomy 8+18 mosaicism at age 7 weeks (panels A-C) and age 1 year (panels D-E).
original blood sample (taken at age 2 days) on the Affymetrix Genome-Wide Human SNP Assay Kit version 6.0 (Affymetrix, Inc., CA). No indication of uniparental disomy (i.e. larger regions of homozygocity) was found, and no additional (and structural) de novo copy number abnormalities were detected.

A re-examination of the original leukocyte cell culture suspension with FISH-probes for centromeres 8 and 18 explained the surprising CGH finding: In 2% of the metaphases (100 metaphases examined) and 15% of the interphases (200 nuclei examined) tetrasomy for both chromosomes was found (Figure 2, panels C and D). The discrepancy between the CGH-findings (50% dose increase for 8 and 18, suggesting that 50% of the cells had four chromosomes 8 and 18) and the cell culture findings (15% of the interphases had four chromosomes 8 and 18) were likely due to negative selection of double tetrasomic cells during PHA-stimulated culturing of blood leukocytes. This illustrates that conventional G-banded karyotyping may easily overlook such mosaicism. To investigate other tissues for similar mosaicism, a skin biopsy from the inside of the left thigh was collected for fibroblast culturing at age 4 months, and at age 14 months a buccal smear was collected. In none of these tissues double tetrasomic cells were found by interphase FISH with 200 nuclei examined in each case. At age 20 months, a new blood sample was taken, and the double tetrasomic cells could no longer be detected, neither by CGH nor by interphase FISH.

Figure 2
Chromosome-based high-resolution CGH result (panel A) and 1 Mb BAC-array CGH result (panel B) on a blood DNA sample collected at age two days. The apparent 50% increase in DNA amount corresponding to chromosomes 8 and 18 was due to double tetrasomy of both chromosomes (panel C, showing a double tetrasomic metaphase). By interphase FISH, four copies of chromosomes 8 and 18 were detected in 15% of the cells from a 3-days PHA-stimulated blood culture (panel D).
To determine the origin of the four extra chromosomes (two chromosomes 8 and two chromosomes 18) that probably were present in around 50% of the blood leukocytes at the time of birth, microsatellite markers for chromosomes 8 and 18 were compared between blood-DNA samples from the parents and the original child blood-DNA sample taken at age 2 days (Table 1). There was no indication of more than two alleles for any simple tandem repeat examined, making meiotic non-disjunction an unlikely mechanism. Furthermore, there was no systematic skewing of the ratios between maternal and paternal allele peak sizes (Table 1), which would have been the case if three of the chromosomes in each quadruple were uniparental. Taken together, this indicates that the mosaicism was a consequence of mitotic events, and that the origin is a mitotic division where all four chromatids of two chromosome pairs (8 and 18) segregated to one daughter cell only.

The finding of tetrasomy for two autosomes in a newborn is without precedence. To the best of our knowledge, even single chromosome tetrasomy mosaicism has not previously been reported – except in cancer cells. The reason for the uniqueness of our finding is either that this indeed is a very rare chromosomal aberration, or that this type of malsegregation commonly occurs but is not detected due to negative selection against aneuploid cells during embryonic development and the culturing of blood cells for routine karyotyping. We were fortunate to observe this because we performed "unbiased" CGH analyses on leukocyte DNA from a newborn. Moreover, extra copies of both of the involved chromosomes are known to be compatible with sustained cell growth in the embryo, increasing the likelihood that a double tetrasomic cell line could survive to term. Notably, the initial cytogenetic investigation (G-banding) appeared normal – only a later reexamination by FISH revealed double tetrasomic cells and metaphases (Figure 2). Apparently, the short-term PHA-stimulated leukocyte culture decreased the number of aberrant cells from around 50% to 15%. Twenty months later the aberrant clone was undetectable in blood, probably because it was counter-selected in the bone marrow.

At a later time point we were unsuccessful in finding double tetrasomic cells in other tissues, i.e. in squamous epithelial cells from a buccal smear and cultivated fibroblasts.

Table 1: Allele sizes of polymorphic chromosome 8 and 18 simple tandem repeats.

| UniSTS | Position (Mb from pter) | Mother (m) | Father (p) | Child | Ratio m/p peak heights |
|--------|-------------------------|------------|------------|-------|------------------------|
| Chrom. 8: | | | | | |
| D8S264 | 21 | 153–155 | 143–153 | 143–155 | 0,50 |
| D8S1104 | 41 | 135–135 | 143–143 | 135–143 | 1,01 |
| D8S268 | 41 | 260–266 | 264–266 | 260–264 | 1,24 |
| D8S531 | 49 | 121–127 | 123–123 | 121–123 | 1,60 |
| G08718 | 49 | 219–219 | 214–214 | 214–219 | 0,94 |
| D8S178 | 53 | 251–253 | 255–258 | 251–255 | 1,37 |
| D8S517 | 62 | 207–215 | 209–215 | 207–215 | 1,05 |
| D8S277 | 65 | 165–173 | 165–169 | 169–173 | 1,18 |
| D8S270 | 93 | 101–112 | 110–112 | 101–110 | 1,84 |
| D8S1784 | 83 | 288–288 | 282–286 | 282–288 | 0,71 |
| D8S550 | 109 | 194–212 | 210–210 | 210–212 | 0,65 |

Mean 1.03

| Chrom. 18: | | | | | |
| D18S452 | 6 | 128–144 | 132–136 | 136–144 | 1,00 |
| D18S53 | 11 | 165–173 | 165–169 | 169–173 | 1,19 |
| D18S453 | 13 | 148–152 | 152–152 | 148–152 | 2,13 |
| D18S71 | 13 | 270–278 | 258–276 | 258–278 | 0,55 |
| D18S61 | 13 | 142–144 | 142–146 | 144–146 | 1,98 |
| D18S104 | 17 | 148–152 | 141–148 | 141–148 | 1,10 |
| D18S1149 | 17 | 258–265 | 263–267 | 263–265 | 0,77 |
| D18S869 | 18 | 186–198 | 186–189 | 186–198 | 1,39 |
| D18S478 | 23 | 248–250 | 246–246 | 246–250 | 1,21 |
| D18S1102 | 33 | 90–94 | 90–92 | 90–94 | 0,97 |
| D18S474 | 47 | 124–126 | 132–138 | 126–138 | 2,68 |
| D18S61 | 66 | 228–230 | 232–232 | 228–232 | 2,13 |
| D18S1161 | 70 | 231–233 | 219–219 | 219–233 | 0,79 |

Mean 1.24

The ratio between the maternal and paternal peak heights is given in the right column, with the geometrical means of each chromosome in bold. The names of the centromeric repeats are written in italics.
from a skin biopsy taken from left groin, where skin pig-
ment mosaicism could be seen. The reason for this, at
least when the fibroblasts are concerned, can be the
unpredictable distribution of mosaicism and not neces-
sarily a negative selection process [10]. Even though we
lack cytogenetic proof that the mosaicism affects other tis-
sues than the bone marrow, the clinical picture suggests
this. The child’s phenotype has elements of resemblance
to children with mosaicism for trisomy 8 or trisomy 18
(Table 2). In fact, there are no known physical features in
the patient, with the possible exception of poorly devel-
oped lower eyelashes, that has not been reported in
patients with trisomy 8 or 18 mosaicism [11-13].

This case is of particular interest because it illuminates
early events in embryogenesis that may have implications
for tumor biology. Our data suggests that all four chroma-
tids of the homologous pair were pulled to one daughter
cell only by the mitotic spindle apparatus, analogous to
the syntelic attachment of the mitotic spindle that may be
seen in tetraploid yeast cells [5]. Unlike other organisms,
pairing of homologous chromosomes in somatic cells is
commonly seen in Dipterians such as Drosophila and mos-
quitos [14]. Notably, homologous pairing in both meio-
sis and mitosis occurs independently of synapsis and
recombination [14]. Furthermore, the finding of (mosaic)
segmental isodisomy as a disease mechanism in some
cases of imprinting-related growth syndromes (e.g. Beck-
with-Wiedemann syndrome and Russell-Silver syndrome,
[15]) also indicates that mitotic homologous pairing takes
place. That such pairing is not limited to imprinted chro-
mosomes are illustrated by the reports of children with
recessive diseases due to homozygocity for mutations car-
ried by one of the parents only, the disease manifesting
due to segmental isodisomy formation [16-19].

We believe that the most likely explanation for the double
tetrasomy is that the starting point was a tetraploid state,
which is the normal situation after S-phase, or a tetraploid
cell line. If the origin was a tetraploid state, aborted S-
phase (mitotic slippage) or interrupted M-phase (failed
cytokinesis) are possible mechanisms. Chromatid non-
disjunction is one suggested reason for failed cytokinesis
[6,8]. If the origin was a tetraploid cell line, there are scant
indications that such cells may later become diploid. In
Candida albicans tetraploid strains become diploid or near
diploid through “concerted chromosome loss” [20]. In
hepatic cells being tetraploid after fusion to bone marrow
stem cells, a “reduction mitosis” appears to be able to
transform tetraploid hybrids into diploids [21]. In both
cases the mechanism is unknown. A variable number of
tetraploid cells is commonly found in chorion villus or
amniocyte cultures, but such cells are rarely found in live-
borns [22]. The origin can be meiotic or mitotic errors
[23,24].

Conceivably, failed cytokinesis or mitotic slippage might
be quite common events in early embryogenesis, for

Table 2: The patient’s phenotypic features compared to cases with mosaic trisomy 8 or 18

|                          | Our patient: Double tetrasomy 8+18 mosaicism | Trisomy 8 mosaicism | Trisomy 18 mosaicism |
|--------------------------|---------------------------------------------|---------------------|---------------------|
| Short stature            | +                                           | +                   |
| Small head               | +                                           | +                   |
| Feeding problems         | +                                           | +                   |
| Developmental delay      | +                                           | +                   |
| Deafness, conductive    | +                                           | +                   |
| High frontal hairline/prominent forehead | +                                   | +                   |
| Low-set/posteriorly rotated ears | +                               | +                   |
| Crumpled ear helices     | +                                           | +                   |
| Narrow/atretic auditory canals | +                                | +                   |
| Middle ear abnormalities | +                                           | +                   |
| Short palpebral fissures | +                                           | +                   |
| Epicantic folds          | +                                           | +                   |
| Downslant                | +                                           | +                   |
| Broad nasal bridge       | +                                           | +                   |
| Thin upper lip           | +                                           | +                   |
| Small chin               | +                                           | +                   |
| Short neck               | +                                           | +                   |
| Skin pigmentation anomalies | +                                  | +                   |
| Overriding toes          | +                                           | +                   |
| Coarctatio aortae        | +                                           | +                   |
| Ventricular septal defect (VSD) | +                              | +                   |
| Persistent ductus arteriosus (PDA) | +                          | +                   |
| Retinitis pigment–like findings in retina | +                        | +                   |
instance during the rapid cell cycles taking place in the peri-gastrulation stage of mammalian embryogenesis [25]. If there exists a special mechanism to deal with this, e.g. to reinitiate the spindle apparatus after the failure has been corrected, balanced segregation would be an advantage, and this could require pairing of the homologues. A further hypothetical advantage of such pairing is removal of replication errors or detrimental mutations from at least one of the daughter cells by segregating both chromatids of one such pair segregates to the same daughter cell. We suggest that this might have happened very early in development to two homologue pairs, made by chromosomes 8 and 18. We also illustrate how a detrimental mutation that has arisen during replication (marked by an asterix) may be eliminated by segregation to one daughter cell only after a mitotic cross-over that also generates a segmental (and terminal) uniparental isodisomy.

Conclusion

This unique case indirectly suggests that a mechanism for tetraploidy correction involving pairing of homologues may be present in somatic cells, and that mosaicism originating in tetraploidization could be a cause of developmental abnormalities that usually remain undetected. When such a mechanism is dysfunctional, aneuploidy is a likely result – which is commonly found in many types of cancer.

Consent

The parents have given written consent to publication of the patient’s pictures, and the parents have also seen and approved the publication of the disease history.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

GH initiated the study, and was the person mainly responsible for its design and conclusions. HL carried out the molecular genetic studies and participated in the writing of the manuscript. SG collected clinical data and participated in the writing of the manuscript. All authors have read and approved the final manuscript.

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