Confined Trisomy 8 Mosaicism of Meiotic Origin: A Rare Cause of Aneuploidy in Childhood Cancer

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Whether chromosome abnormalities observed in tumor cells may in some cases reflect low-grade somatic mosaicism for anomalies present already at zygote formation, rather than acquired somatic mutations, has for long remained a speculation. We here report a patient with Wilms tumor, where constitutional somatic mosaicism of trisomy 8 was detected in a previously healthy 2 ½-year-old boy. Single Nucleotide Polymorphism (SNP) array analysis of tumor tissue revealed a complex distribution of allele frequencies for chromosome 8 that could not be explained solely by mitotic events. Combined analysis of allele frequencies, chromosome banding, and fluorescence in situ hybridization revealed that the majority of tumor cells contained four copies of chromosome 8, with three distinct haplotypes at a 2:1:1 ratio. Because the patient had not been subject to organ transplantation, these findings indicated that the tumor karyotype evolved from a cell with trisomy 8 of meiotic origin, with subsequent somatic gain of one additional chromosome copy. Haplotype analysis was consistent with trisomy 8 through nondisjunction at meiosis I. Matched normal renal tissue or peripheral blood did not contain detectable amounts of cells with trisomy 8, consistent with the complete lack of mosaic trisomy 8 syndrome features in the patient. This case provides proof of principle for the hypothesis that tumor genotypes may in rare cases reflect meiotic rather than mitotic events, also in patients lacking syndromic features.

INTRODUCTION

Mosaic trisomy 8 is a rare condition with prevalence estimates in the range of 1:25,000–1:50,000 births and a preponderance in males. The condition is clinically heterogeneous, and it is associated with a wide array of different developmental abnormalities, including mental retardation and congenital heart defects (Jordan et al., 1998; Wisniewska and Mazurek, 2002). Approximately 50% of trisomy 8 mosaicism syndrome patients present with renal abnormalities (reviewed in Aykut et al., 2012). A case of bilateral cystic nephroblastoma in a patient with mosaicism for trisomy 8 has also been reported (Nakamura et al., 1985). It is estimated that the majority of mosaic trisomy 8 cases are due to early mitotic errors, implying that mosaic trisomy 8 of meiotic origin typically does not lead to live birth (Karadima et al., 1998).

Trisomy 8 is also well known to be present as the sole anomaly in various neoplasms, particularly in acute myeloid leukemia and myelodysplastic syndromes, and it is generally agreed that the extra chromosome 8 in this context is acquired. However, constitutional trisomy 8 mosaicism (CT8M) occur in approximately 0.1% of all recognized pregnancies which suggests that trisomy 8 ascertained in leukemias may in some cases be constitutionally derived (reviewed in Paulsson and Johansson, 2007). In fact it has been proposed that CT8M could predispose to hematologic neoplastic disorders (Maserati et al., 2002).

Here we report an unexpected case of mosaic trisomy 8, in a context of several genomic imbalances revealed by SNP-array analysis of a Wilms tumor from a 2 ½-year-old boy. Surprisingly, the distribution of genotypes, based on B Allele Frequencies (BAF), for chromosome 8 in the tumor sample, indicated that there was an extra haplotype present, implying a trisomy of meiotic origin. This extra haplotype was not present in the matched normal kidney sample. Fluorescent in situ hybridization (FISH) for chromosome 8, as

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TABLE 1. Genetic Aberrations Detected by SNP Array in the Tumor of the Patient

Chromosome	Start	End	Aberration
6	0	170912503	Gain
8	0	146364022	Gain
9	70984372	96238218	Gain
9	96242455	104846881	Loss
9	104863013	141022295	Gain
12	0	133851895	Gain

Positions are given according to hg19.

well as G-banding of both tumor and normal tissue suggested a vanishing mosaicism for trisomy 8.

MATERIAL AND METHODS

Patient/Subject

The patient, a 2 1/2-year-old boy, presented with macroscopic hematuria after abdominal trauma. Clinical and radiological examinations revealed a tumor in the right kidney and the patient started treatment as specified by the SIOP-2001 protocol. Histopathologic analysis of the tumor-nephrectomy specimen showed a stage I Wilms tumor of stromal type, with a single perilobar nephrogenic rest. Material from the nephrectomy, containing tumor material as well as normal kidney, was preserved at −80°C. The patient had normal psychomotor development and lacked genitourinary or other malformations. The study had been approved by the regional ethics committee (ref. no. L119-03) Lund, Sweden.

SNP-Array

Tumor DNA was extracted from frozen tumor tissue using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA), and 300 ng was hybridized to the 1M Genotyping BeadChip SNP-array HumanOmni1-Quad (Illumina Inc., San Diego, CA), consisting of approximately 1 million genomic markers with a median spacing of 1.5 kb. Genetic aberrations were detected by visual inspection, using the GenomeStudio software V2011.1 (Illumina).

Touch Preparations

Frozen tumor and renal tissue samples were immediately put on dry ice and a 2–3 mm piece was carved from each sample using a scalpel. The carved-off pieces were gently pressed on to microscopy slides, resulting in monolayers of adherent cells. The cells were treated with one part of acetic acid and three parts of methanol for 10 min, then air dried and stored at −20°C prior to analysis by FISH.

FISH and Chromosome Banding

Interphase FISH was performed as previously described (Gisselsson, 2001). Chromosome enumeration probes for chromosome 8 (CEP8 Spectrum Green) as well as for control chromosomes (chromosome 10 and chromosome 9; CEP10 Spectrum Orange, CEP9 Spectrum Orange) were obtained from Abbott Molecular (Abbot Park, IL). Cytogenetic analyses were performed by routine methods, with Wright's stain used to obtain G banding. At least 25 cells were analyzed cytogenetically per sample.

RESULTS

SNP array analysis of the Wilms tumor sample showed a genetic profile typical of Wilms tumor with whole chromosome gains of 8 and 12, segmental gains of chromosomes 6 and 9, and segmental loss of chromosome 9 (Table 1). No genetic aberrations were detected by SNP array analysis of normal kidney. In the tumor sample, BAF values for chromosome 8 formed five tracks over several segments at about 0; 1; 0.75; 0.25; and 0.5 (Fig. 1). This complex B Allele Frequency (BAF) profile suggested the presence of at least three haplotypes (Jinawath et al., 2011), whereof one homologue is a der (8) with a partly different haplotype than the other two homologues, suggesting a meiosis I error (Supporting Information Fig. 1). To ascertain the copy number status of chromosome 8, we performed interphase FISH on touch preparations from the tumor, which showed a tetrasomic stemline (60%, 71/119 cells) and a minor clone carrying trisomy 8 (24%, 28/119 cells). FISH for the same chromosome on peripheral blood smears and on normal renal tissue showed disomy 8, without an elevated fraction of cells with three signals compared to background levels of control chromosomes. G-banding of tumor tissue revealed a hyperdiploid karyotype of 50, XY, +6, +8, +8, and +12, that is, tetrasomy for chromosome 8 (Fig. 2). Only one of the 25 analyzed cells was trisomic for chromosome 8 (Fig. 2). G-banding of peripheral blood revealed a normal male karyotype (46, XY).

DISCUSSION

The fact that three chromosome 8 haplotypes were present around the centromeres in the

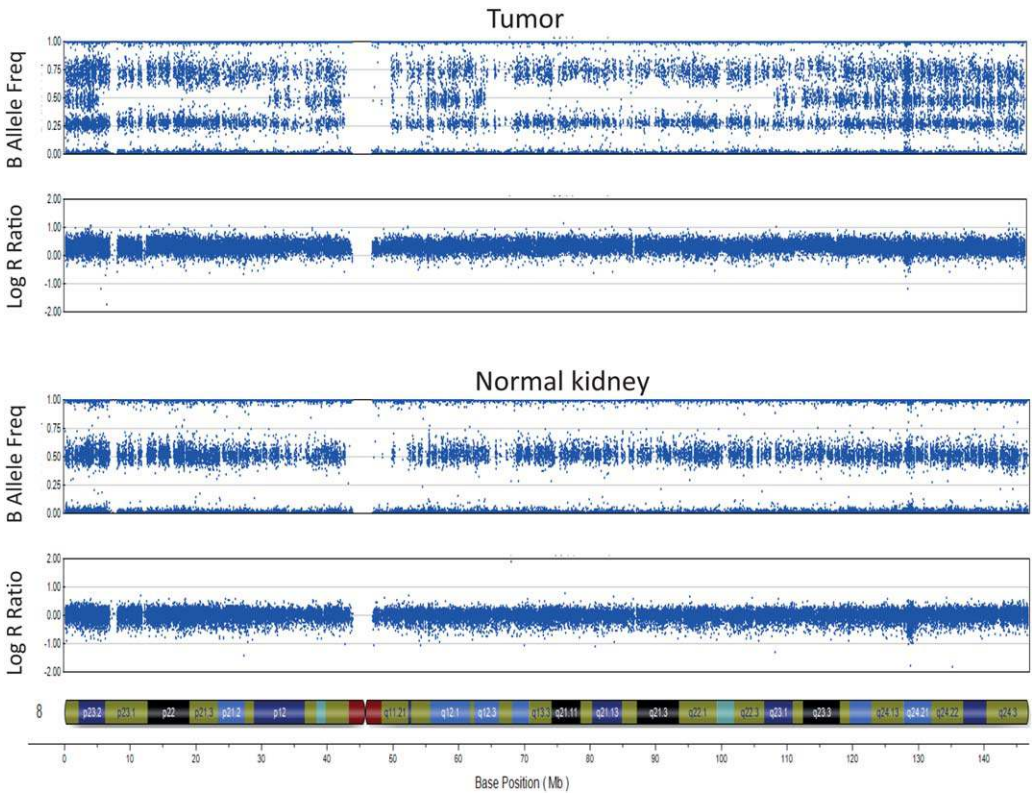

Figure 1. SNP array profiles of chromosome 8 of tumor as well as normal renal tissue. Figure created in Genome Studio (Illumina). Log R ratio corresponds to copy number. The B Allele Frequency (B Allele Freq) shifts between 5 and 4 tracks, which illustrates the presence of more than two different homologues. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

tetrasomic tumor clone (represented by SNP array BAF values) suggests a meiosis I error, as a meiosis II or mitotic error would not result in more than two haplotypes in the centromeric/pericentromeric region (Conlin et al., 2010). A trisomy 8 cell (containing haplotypes from gamete A in Fig. 2) would then represent the lineage of origin of the Wilms tumor clone, whereas mitotic nondisjunction would result in the disomic cell clone, making up the bulk of cells of the individual. Thus, the normal karyotype found in normal kidney and peripheral blood would contain acquired disomy 8 (Fig. 2). One might envision that the trisomic clone could have impaired ability to divide and would linger in the kidney tissue until additional genetic aberrations would trigger proliferation and tumor onset. Although rare, according to literature, early correction of a postzygotic trisomic clone has previously been discussed as a potential explanation for trisomic tumor cell populations (Haas and Seyger, 1993). Such trisomic corrections, where one chromosome is lost early during pregnancy, bring the risk of uniparental disomy (UPD) as a consequence of nondisjunction of the trisomic cell clone (Fig. 2, potential UPD clone not depicted). SNP array profile of chromosome 8 in normal kidney showed no UPD in the present case (Fig. 1).

The degree of somatic mosaicism for a certain chromosome anomaly is notoriously hard to establish and correlate to clinical features (Chandley et al., 1980). Fibroblast cultures from a skin biopsy could have given additional information about tissue restriction of the mosaicism in this individual (Niss and Passarge, 1976). However, such sampling was not considered clinically relevant and thus not ethically motivated for the patient, as he did not suffer from any phenotypic characteristics associated with mosaicism for trisomy (or tetrasomy) 8. There have been some earlier case reports of disappearing mosaicism (Mark and Bier, 1997), where the growth advantage of karyotypically normal cells over the trisomic clone results in a diminishing mosaic clone (La Marche et al., 1967; Hulley et al., 2003). Possibly, this is also the case for our patient, corroborated by the fact that we did not detect any trisomic cells in peripheral blood through either classic cytogenetics or interphase FISH. The lack of a trisomic clone in the

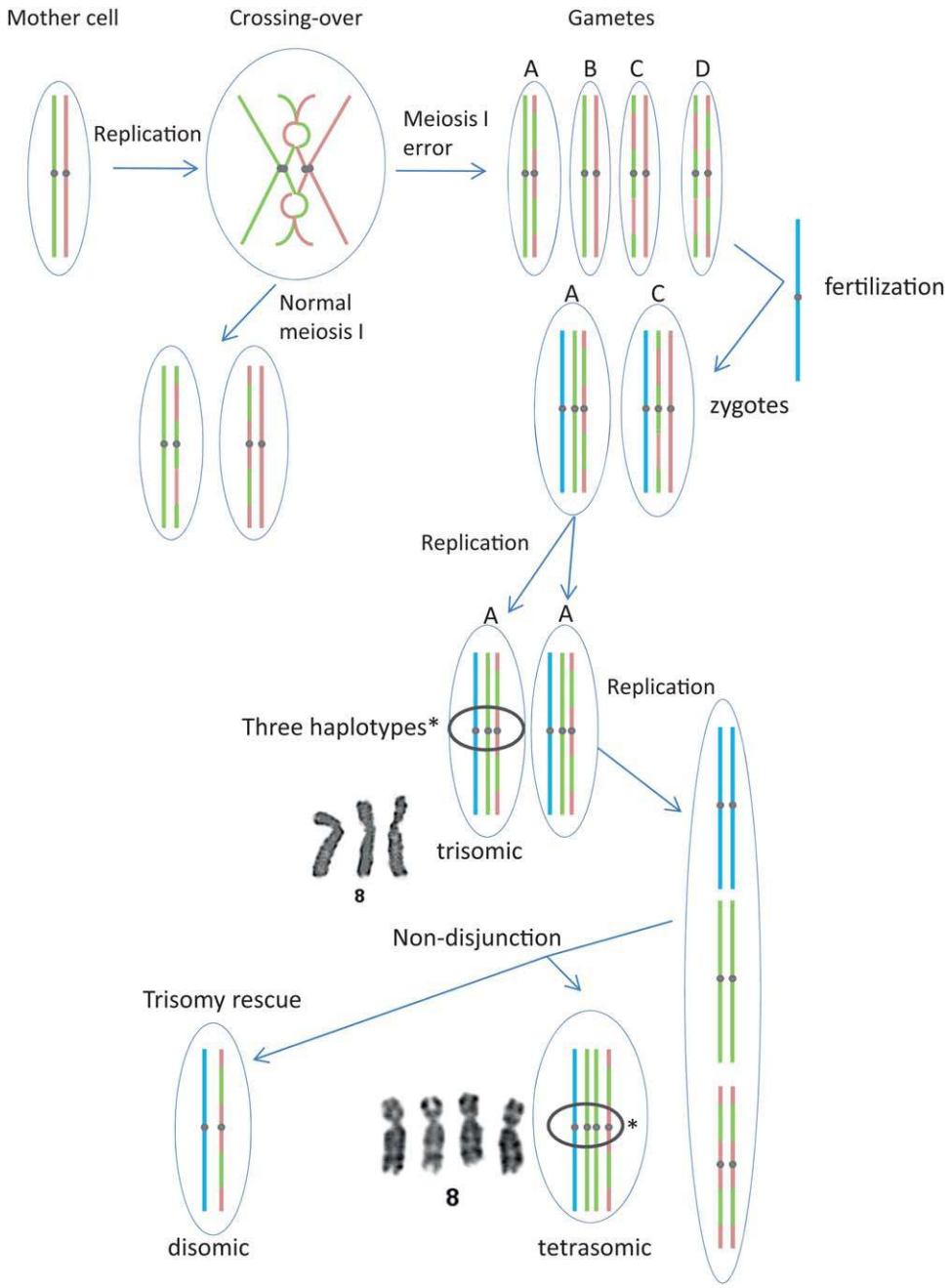

Figure 2. Mosaic trisomy 8 caused by nondisjunction in meiosis I followed by mitotic nondisjunction postzygotically. Only chromosome 8 is shown. The diploid mother cell is replicated and the homologous chromosomes synapse and cross overs take place. In our mosaic tumor case nondisjunction takes place at meiosis I resulting in four aneuploid gametes which upon fertilization of either gamete A or C by a normal haploid external gamete results in chromosome 8 trisomic zygotes which represent three haplotypes (asterisk in figure). One possible explanation for the presence of both a tetrasomic (shown by chromosome banding of subject tumor tissue) and a trisomic (shown by chromosome banding of subject tumor tissue) chromosome 8 clone could be a second event of mitotic nondisjunction early in the development in conjunction with trisomy rescue to obtain the disomic clone which would make up the major population of the mosaic individual. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

normal renal samples analyzed also substantiates this. In a previous report on a Wilms tumor patient with CMT8, the patient was both mentally and physically severely disabled. Growth of his lymphocytes revealed a diploid karyotype, but growth of cultured fibroblasts revealed mosaicism for chromosome 8 trisomy. Thus, the trisomy 8 clone was restricted to fibroblasts and with increased number of subcultures the trisomic clone diminished. The origin of the mosaicism, and whether it was present in tumor cells, was not determined (Niss and Passarge, 1976).

The frequency of constitutionally derived trisomy 8 in leukemia and other neoplasms is not known, but case reports show their existence (Seghezzi et al., 1996; Paulsson and Johansson, 2007). Little is known of the origin of these CT8M, possibly due to the fact that researchers have not investigated available samples to differentiate CT8M of mitotic and meiotic origin. To the best of our knowledge, there is so far no report showing a CT8M as a consequence of a meiosis I error. The only reported case of constitutional trisomy mosaicism similar to our case is one of erythroleukemia, in which a constitutional trisomy 21 mosaicism was discovered. The patient showed no apparent signs of Down syndrome but a trisomic clone of chromosome 21 was found in the patient's bone marrow at disease presentation. By microsatellite analysis, it was conveyed that the trisomic clone was derived from a maternal meiosis I error, but it could not be proved unequivocally to what extent the neoplastic cells contained extra copies of the meiotically derived extra chromosome 21 (Minelli et al., 2001).

In summary, the present case shows that, in rare cases, aneuploidy detected solely in tumor tissue may in fact result from meiotic errors and suggests that such rare situations of confined mosaicism may predispose to tumor formation in nonsyndromic patients.

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