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Meiotic origin of trisomy in neoplasms: evidence in a case of erythroleukaemia

A Minelli[1], C Morerio[2], E Maserati[3], C Olivieri[1], C Panarello[2], L Bonvini[3], A Leszl[4], C Rosanda[2], E Lanino[2], C Danesino[1] and F Pasquali[3]

[1]*Biologia Generale e Genetica Medica, Università di Pavia, Pavia;* [2]*Divisione di Ematologia ed Oncologia Pediatrica, Istituto Giannina Gaslini, Genova;* [3]*Sezione di Biologia e Genetica, Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Università dell'Insubria, Varese; and* [4]*Divisione di Oncoematologia Pediatrica, Dipartimento di Pediatria, Università di Padova, Padova, Italy*

Trisomic cells in neoplasms may represent abnormal clones originated from a tissue-confined mosaicism, and arise therefore by a meiotic error. We report on a 16-month-old child with erythroleukaemia (AML-M6), whose marrow karyotype at onset was 48,XX,del(13)(q12q14),del(14)(q22q32),+21,+21. The parental origin of the supernumerary chromosomes 21 was investigated by comparing 10 polymorphic loci scattered along the whole chromosome on the patient's marrow and her parents' leukocytes. Three loci were informative for the presence of three alleles, two of which were of maternal origin; two further loci showed a maternal allele of higher intensity. Lymphocytes and skin fibroblasts showed a normal karyotype, and molecular analysis on leukocytes at remission, buccal smear and urinary sediment cells consistently showed only one maternal allele, whereas neonatal blood from Guthrie spot showed two maternal alleles as in the marrow. An accurate clinical re-evaluation confirmed a normal phenotype. Our results indicate that tetrasomy 21 arose from a marrow clone with trisomy 21 of meiotic origin. To the best of our knowledge, this is the first evidence that supernumerary chromosomes in neoplastic clones may in fact be present due to a meiotic error. This demonstrates that a tissue-confined constitutional mosaicism for a trisomy may indeed represent the first event in multistep carcinogenesis. *Leukemia* (2001) **15,** 971–975.
Keywords: trisomy 21; tetrasomy 21; erythroleukaemia; AML-M6; meiotic non-disjunction

Introduction

Trisomies are frequent in neoplasms: they often are the only chromosome anomaly present at onset in neoplastic clones,[1] and are therefore regarded as primary changes in their pathogenesis. This is true in particular for haematological malignancies, in which some trisomies are so frequent in a disease or a disease phase, that they certainly play a specific pathogenetic role:[2] this is the case of trisomy 8 in acute myeloid leukaemias (AML) or trisomies 8 and 19 in the blastic phase of chronic myelocytic leukaemia (CML). In 1993, Haas and Seyger[3] postulated that some of these trisomies represent evidence of tissue-confined residual cell populations of meiotic origin. This hypothesis was based on circumstantial evidence, and raised some interest,[4–6] but it was never supported by any direct evidence.

We report on a child with erythroleukaemia associated with tetrasomy 21, in which microsatellite analysis gave evidence of the meiotic origin of one supernumerary chromosome 21 in the marrow, supporting the hypothesis mentioned above, and in the absence of clinical signs of Down syndrome (DS). We failed to obtain evidence of a trisomic cell population with cytogenetic and molecular methods in other available tissues, except for neonatal blood spots.

Correspondence: E Maserati, Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Università dell'Insubria, Via JH Dunant 5, I 21100 Varese, Italy; Fax: ++39–0332–217119
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Case report

PF, a female, was born in April 1997 after 39 weeks of gestation from healthy, unrelated parents. Her birth weight was 2880 g, her length 49 cm, Apgar score 10 at 1 min, 10 at 5 min. Myopia was diagnosed at 6 months of age. She began to walk at 13 months of age, and to speak at 14. In August 1998, when examined because of persistent fever and pallor, her blood test showed: Hb 6.6 g/dl, WBC 23000/mm^3 with 37% erythroblasts, platelets 40000/mm^3. Her liver and spleen were both palpable 4 cm below the costal margin, and a moderate cervical lymphadenopathy was present. In her bone marrow (BM) the erythroid component was 75% of all nucleated cells, and blasts were 67% of non-erythroid cells. These, resembling atypical proerythroblasts, were large with regular round nuclei, fine lacey chromatin with nucleoli, abundant basophil and non-granulated cytoplasm, sometimes with blebs. They were 12% PAS+, CD34, 33, 117, 13, 45, 38 positive, with a DNA index of 1. A diagnosis of AML of the M6 FAB-type was made, and therapy was started with a standard protocol, including idarubicin, cytosine arabinoside, and etoposide. Complete remission was obtained, and on 27 January 1999 the child underwent a bone marrow transplantation (BMT) from a compatible unrelated donor: the post-transplant course was fairly uncomplicated, and immune suppression was discontinued 11 months after BMT. Full donor-origin haematopoiesis and leukaemia remission were monitored by means of cytogenetics and DNA polymorphism analysis.

In October 1999, she was re-evaluated due to the finding of the constitutional origin of the trisomic 21 marrow clone. She was 91 cm tall (between the 50th and 75th percentile), weighed 14 kg (75th percentile), with a bone age of 28 months (it was 18 months at BMT, when anagraphical age was 21 months). An accurate search failed to reveal any possible DS signs, except for monolateral epicanthal fold associated with a horizontal slant of eyes, small nose with low nasal bridge, severe myopia (–10 D), overfolding upper helix with small earlobe (Figure 1). Milestones in psychomotor development had been reached regularly, and she was considered normal by neuropsychiatrists. EEG, cerebral CT, and abdomen echoscan had been performed in 1998, at AML onset, with normal results except for organomegaly. Hand dermatoglyphics were not as expected in DS: the axial triradius was in *t* and *t'* position in the left and right hand, respectively; there was no excess of loops on fingertips, but three arches and two ulnar loops in the left hand, and one whirl, one arch, one ulnar and two radial loops in the right one.

Methods

Cytogenetic studies

Chromosome analyses were performed repeatedly by routine techniques on BM direct preparations and cultures, on PHA-

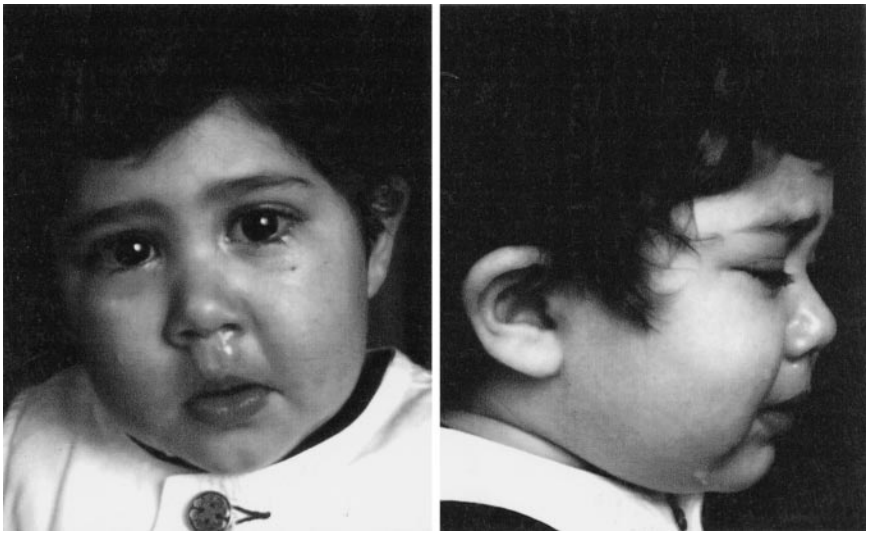

Figure 1 The proband, aged 2 years 6 months.

stimulated peripheral blood (PB) cultures, and on fibroblast cultures from a skin biopsy. Chromosome analysis of both parents and of maternal grandparents was performed on PHA-stimulated PB cultures. QFQ-banding technique was applied.

Fluorescent *in situ* hybridization (FISH) was carried out at onset with whole chromosome paint (Vysis, Downers Grove, IL, USA) for chromosomes 13 and 14 on BM metaphases, with a standard procedure. On BM interphase nuclei we used chromosome 7 and 8-specific alphoid centromeric probes (Vysis), and the unique sequence probe LSI 21 (Vysis) which maps to the chromosome region 21q22.13–21q22.2, according to the manufacturer. In order to obtain evidence of the constitutional trisomy 21 mosaicism, FISH analysis on interphase nuclei was then performed with the LSI 21 probe also on PHA-stimulated blood cells, and on skin fibroblasts. Control slides from healthy subjects were concurrently used to assess the significance of the results obtained on nuclei. All the images were obtained through a Zeiss Axiophot 2 epifluorescence microscope (Carl Zeiss, Jena, Germany) equipped with a CCD camera, and analyzed by PowerGene Systems (Perceptive Scientific Instruments, Chester, UK).

Molecular studies

Molecular studies were performed to gather information on the parental origin of the chromosome(s) 21 involved in tetrasomy. The loci tested, scattered along the whole chromosome, were 10, and four more loci on different chromosomes were tested to confirm paternity. We extracted DNA with routine techniques from BM sampled on 27 August 1998, PB sampled on 16 October 1998, Guthrie spots, buccal smear and urinary sediment cells of the patient, and from her parents' blood samples. Genotyping of STRs (Research Genetics, Huntsville, AL, USA) was performed by use of standard procedures. PCR amplifications were performed in 8 μl reaction mixtures containing 20 ng genomic DNA; 330 nM of each primer; 200 μM of dCTP, dGTP, dTTP and dATP; 50 mM KCl; 10 mM TRIS pH 9; 1.5 mM $MgCl_2$; 0.1% Triton X-100; 0.01% gelatine and 0.2 U of *Taq* Polymerase. The PCR conditions consisted of an initial denaturation step followed by 30 cycles of 94°C for 40 s, 57°C for 40 s, 72°C for 40 s and a final extension at 72°C for 5 min, using a PTC-100 thermal cycler (MJ Research, Waltham, MA, USA). Four microliters of each PCR product were resolved by electrophoresis on denaturing (7M urea) 7% polyacrylamide gels for 1–3 h at 30 W. Gels were fixed in 10% methanol/acetic acid, and stained with silver nitrate 0.012 M.

Results

Table 1 summarizes the results of chromosome analyses performed, and Table 2 the FISH data obtained on interphase nuclei to detect trisomic 21 cell populations. At onset, an abnormal clone was found in most BM mitoses, with tetrasomy 21 and apparently interstitial deletions of chromosomes 13 and 14. Painting for these latter failed to detect material transposed elsewhere, thus confirming the interpretation of the derivative chromosomes as simply deleted. Interphase nuclei analysis showed normal disomy for chromosomes 7 and 8, whereas tetrasomy 21 was confirmed in 181 nuclei out of 215 examined (Table 2). Out of 55 mitoses analyzed three presented with a normal karyotype, and only one with trisomy 21 (Table 1).

After remission was achieved, the BM karyotype became normal, as was in PHA-stimulated PB cultures. Fibroblast cul-

Table 1 Results of chromosome analyses

Date	*Material*[a]	*Karyotype*
27.08.98	BM	47,XX,+21[1]/48,XX,del(13)(q12q14), del(14)(q22q32),+21,+21[51]/46,XX[3]
01.09.98	BM	48,XX,del(13)(q12q14),del(14)(q22q32), +21,+21[20]/46,XX[1]
08.10.98	BM	46,XX[33]
16.10.98	PB-PHA	46,XX[67]
07.01.99	BM	47,XX,+21[2]/46,XX[17]
22.02.99	BM	46,XY[42]
12.04.99	BM	46,XY[38]
08.07.99	SF	46,XX[101]

[a]BM, bone marrow direct preparations and 24–48 h cultures; PB-PHA, PHA-stimulated peripheral blood culture; SF, skin fibroblast culture.

Table 2 Results of interphase nuclei FISH with the chromosome 21 probe LSI 21 (Vysis), which maps to the chromosome region 21q22.13–21q22.2

Date/Material[a]	*No. of nuclei (%) with*				
	0 signal	*1 signal*	*2 signals*	*3 signals*	*4 signals*
27.08.98 BM	—	—	31 (14.4)	3 (1.4)	181 (84.2)
16.10.98 PB-PHA	—	—	106 (96.4)	4 (3.6)	—
08.07.99 SF	—	5 (0.8)	570 (95.0)	11 (1.8)	14 (2.3)
Control 1	2 (0.4)	16 (3.5)	440 (95.4)	2 (0.4)	1 (0.2)
Control 2	1 (0.2)	11 (2.4)	440 (97.1)	1 (0.2)	—
Control 3	1 (0.5)	10 (4.7)	201 (94.4)	1 (0.5)	—
Control 4	—	7 (3.4)	197 (94.7)	3 (1.4)	1 (0.4)

[a]Abbreviations as in Table 1.

tures from a skin biopsy also showed a normal karyotype (Table 1). Trisomy 21 was found again only in two mitoses from the BM analysis performed just before the BMT, in January 1999. In addition, four other cells with three chromosomes 21, but with random losses of other chromosomes, had also been observed in the BM analysis made in October 1998.

Further interphase studies failed to reveal a trisomic 21 cell population both in preparations from PHA-stimulated PB cultures and from skin fibroblast cultures (Table 2). In fact, we compared the results with those obtained in four healthy controls, where the proportion of cells with less than two signals was in the range 2.6–5.2%, and that of cells with more than two signals in the range 0.2–1.8% (Table 2). Cells with two signals consistently represented in controls at least 94.4% of the total (Table 2). The proportion of cells with three and four signals found in our patient's PHA-stimulated PB and fibroblasts might look different from controls, but small numbers and slightly variable results in controls are sufficient to give this impression. To draw unbiased conclusions we compared by chi-square the frequency of cells with more than two signals in our patient's stimulated blood and fibroblasts with that of control 4 (Table 2), and the differences were found not to be significant ($P < 0.25$ and $P < 0.10$, respectively).

The chromosome analyses performed after BMT (January 1999) documented a good engraftment. The parents' karyotypes were normal, as were those of maternal grandparents.

Analysis of the STRs was informative in five out of 10 tested (Table 3): in the BM sample three loci (D21S1413, D21S1414, D21S2055) showed three alleles, two of which were of maternal origin (Figure 2), while two loci (D21S1436, D21S1437) showed only two alleles, with the maternal one of higher intensity as compared to the paternal (Figure 2). Three alleles were also seen in the DNA sample obtained from the Guthrie spot at loci D21S1413 (Figure 2) and D21S2055. The PB sample consistently showed two alleles without any evident difference of intensity (Figure 2), as did the cells obtained from buccal mucosa. Cells from the urinary sediment showed the same two alleles as in blood at locus D21S1413, and a third allele similar to a paternal one at locus D21S2055: as these cells were obtained after BMT, and a small amount of WBC is frequently present in the urinary sediment, we interpreted the third allele as being derived from the donor BM.

Densitometric analysis performed on the lanes of loci with three alleles in BM, showed that the maternal allele not present in blood was of lower intensity with respect to the other two which appeared similar.

The same analysis at loci D21S1436 and D21S1437 on BM, where two alleles were present, confirmed the maternal one as being more intense.

The results obtained in STRs analysis are consistent with a maternal meiosis I error, as is the case in the vast majority of DS patients,[7] with one crossover between D21S2055 and D21S1889 (Table 3). As the aim of our work was not to determine if a meiosis I or II non-disjunction had taken place, the choice of loci used was not instrumental to this issue.

Table 3 Results of microsatellite analysis on father (F), proband's peripheral blood at remission (PB), bone marrow at onset (BM), Guthrie spot (GS), buccal smear (BS), urinary sediment cells (US), mother (M)

Locus	*cM*	*F*	*PB*	*BM*	*GS*	*BS*	*US*	*M*
D21S1414	9.72	BD	AD	**ACD**	—	—	—	AC
D21S1437	13.05	BC	AC	***A***C	—	—	—	AA
D21S1436	13.05	AA	AB	A***B***	—	—	—	BB
D21S1250	17.67	AC	AB	AB	—	—	—	AB
D21S1442	24.73	AB	AA	AA	—	—	—	AA
D21S1270	27.40	AA	AA	AA	—	—	—	AA
D21S1413	29.78	AB	BC	**ABC**	**ABC**	BC	BC	AC
D21S2055	40.49	AB	BC	**BCD**	**BCD**	BC	(A)BC	CD
D21S1889	45.26	AC	AA	AA	—	—	—	AB
D21S1903	53.64	BC	BD	BD	—	—	—	AD

Informative results are shown in bold; three letters refer to the presence of three alleles, alleles of higher intensity are identified by bold italic. The allele A of D21S2055 in brackets in the US column was interpreted as from the marrow donor.

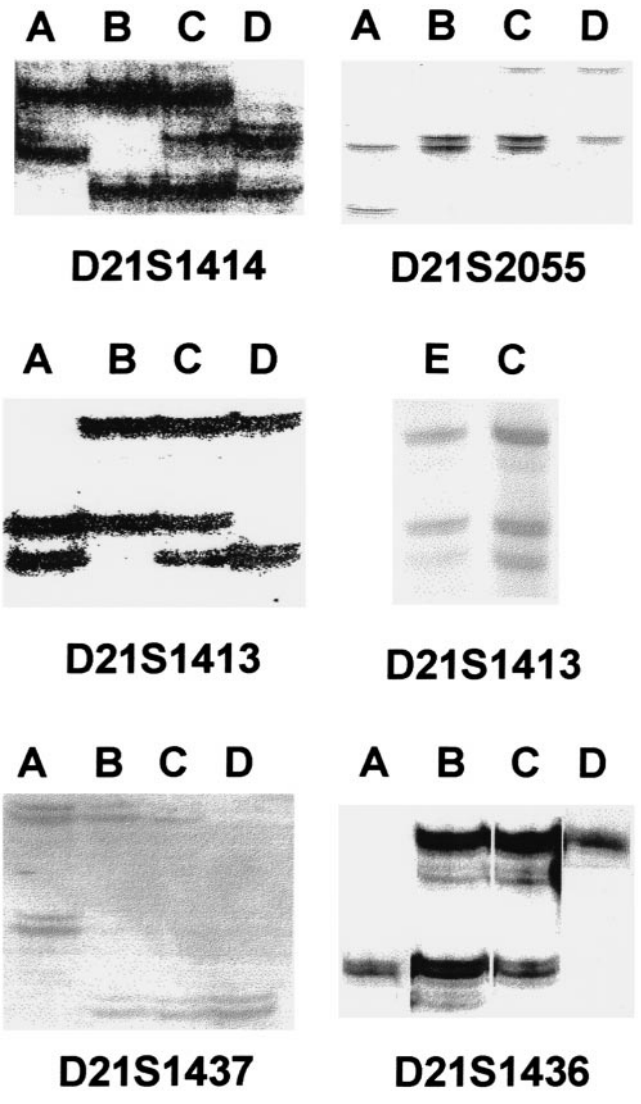

Figure 2 Results of microsatellite analyses at the five informative loci. A, father; B, proband's peripheral blood at remission; C, proband's bone marrow at onset; D, mother; E, Guthrie spot.

Discussion

Trisomy 21 is frequent in AML-M6. It may be regarded as the second most frequent anomaly, after trisomy 8, as 47 out of 390 cases included in the catalogue of chromosome aberrations in cancer[8] showed trisomy 21: it was constitutional in 10. Tetrasomy 21 is less frequent, as the same catalogue lists five cases, four of which had a constitutional trisomy, and also one of pentasomy 21. Tetrasomic (and pentasomic) 21 clones are likely to originate from the trisomic ones by clonal evolution, in particular, when the two cell populations coexist in the marrow, as in the case reported here.

While studying by microsatellite analysis the parental origin of the supernumerary chromosomes 21 in the BM of our case, we found that three loci were consistently informative for the presence of three alleles, and, when only two alleles were present, one was more intense (Table 3, Figure 2). The same uneven intensity of the two alleles was not obvious at two other loci (D21S1250, D21S1903) (Table 3). Our finding of two maternal alleles at least at five loci is consistent only with the origin being a meiotic error, which took place in the mother and led to trisomy 21 in the zygote. Then this constitutional trisomy was corrected, but with the remainder of a trisomic clone at least in BM; this gave origin to the leukaemic clone in which tetrasomy was present, together with additional structural anomalies. To the best of our knowledge this is the first evidence that supernumerary chromosomes in neoplastic clones may originate from trisomic cells of meiotic origin.

The densitometric results obtained on STRs confirmed the higher intensity of one band in BM for loci D21S1436 and D21S1437, which showed only two alleles. As to loci with three alleles, the expected result in a homogeneous cell population, assuming a consistent origin of the tetrasomy from the same maternal chromosome 21, was a 2:1:1 ratio. This was not observed since two bands were evenly intense and stronger than the other one (Figure 2). A possible explanation is that DNA had been extracted from the whole BM sample, where a mixed population of disomic, trisomic and tetrasomic cells is present in proportions difficult to assess.

Our patient had to be considered a constitutional mosaic for trisomy 21, and we tried to demonstrate trisomic cell populations out of BM with conventional cytogenetic methods, using approximately the same standards, as to the number of mitoses to score, which were used to obtain evidence of mosaicism in DS parents.[9] We then added FISH analysis on interphase nuclei, and microsatellite analysis on tissues other than BM. Our efforts failed to obtain any further evidence of trisomy 21, the only exception being the Guthrie spot:

- PB PHA-stimulated cultures gave normal results at chromosome analysis (Table 1): the number of metaphases scored was sufficient to exclude a mosaicism with at least 5% trisomic cells with 0.95 confidence.[10] The disomy 21 was confirmed by interphase nuclei FISH (Table 2), and by microsatellite analysis (Table 3, Figure 2).
- Skin fibroblasts were normal at chromosome analysis (Table 1): the data shown here exclude a 3% mosaicism at 0.95 confidence level.[10] Interphase study confirmed disomy 21 (Table 2).
- Buccal smear cells were found not to be trisomic at microsatellite analysis (Table 3).
- Urinary sediment cells were normal at microsatellite analysis (Table 3).
- PB cells sampled at birth, obtained from a Guthrie spot, showed three alleles at microsatellite analysis, as in BM (Table 3, Figure 2).

The presence of trisomy 21 in blood at birth, as indicated by the results in Guthrie spot, has two possible interpretations. The first one is backtracking the erythroleukaemia of our case to birth: this possibility has already been demonstrated in acute lymphoblastic leukaemia by Wiemels *et al*[11] who showed the presence of TEL-AML1 fusion in neonatal blood from Guthrie spots of six children, who developed the disease 2 or more years later. Alternatively it is evidence of the constitutional mosaicism subsequently demonstrable only in BM (Tables 1, 3). Many cases of trisomy 21 mosaicism arise by postzygotic loss of one chromosome 21,[12] and the proportion of disomic cells may increase with age due to the same mechanism.[13]

We re-evaluated accurately the child phenotype as to DS, even if no suspicion of DS had ever been raised. This re-evaluation took place in October 1999, when the patient was $2\frac{1}{2}$ years old, and did not show an obvious DS phenotype, with a normal physical and psychomotor development. Only a few

possible DS signs were present at physical examination: a small monolateral epicanthal fold, but with a horizontal slant of eyes, small nose with low nasal bridge, and a severe myopia, diagnosed when she was 6 months old, and now at –10 D level.

We provide the first evidence that Haas and Seyger's hypothesis[3] on the meiotic origin of neoplastic clones with trisomies is tenable, as AML-M6 in the present case arose from marrow cells with trisomy 21 of meiotic origin. It is worthy recalling four cases in the literature in which trisomy 21 was detected in marrow, but also in skin fibroblasts, in phenotypically normal infants with a transient myeloproliferative disorder (TMD) of the DS type.[14] These were in fact constitutional mosaics with the trisomy confined to some tissues, and their TMD arose from a trisomic cell, although no molecular evidence was provided to demonstrate a meiotic origin of the trisomy. Bhatt *et al*[14] also reviewed 10 similar cases in which no evidence of trisomy 21 in fibroblasts was achieved, and suggested that some of these might also be true mosaics.

We cannot establish the frequency with which the origin of trisomy in tumours is in fact meiotic, as no similar evidence was available until now, but also evidence of cases in which a meiotic origin was searched for and not found is lacking. However, we demonstrated that a meiotic origin may take place, and further studies on the extension of this phenomenon are warranted because of both theoretical and practical implications, including reproductive genetic counselling. In fact, we may now state that in multistep carcinogenesis the first mutation may also be: (1) a homogeneous trisomy, as of chromosomes 13, 18, or 21 (15); (2) a constitutional evenly distributed trisomy mosaicism, as of chromosomes 8, 9 and 21 (15, 16); (3) a tissue-confined trisomy from meiotic error, as of chromosome 21 in the case reported here.

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