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Tetrasomy 18p Caused by Paternal Meiotic Nondisjunction

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Recently published studies on the etiology of additional monocentric isochromosomes 18p [i(18p)] indicate that the majority of cases are the result of a nondisjunctional event in maternal meiosis II (MII) immediately followed by a centromeric misdivision in meiosis or in an early postzygotic mitosis [1–3]. These findings are consistent with observations in trisomy 18 in which the majority of the reported cases originate from maternal meiosis II [4, 5].

So far, molecular studies on 19 patients with an additional monocentric i(18p) have been reported. Among these, only maternal chromosomes 18 were involved in isochromosome formation. In the majority of cases, a maternal MII nondisjunction was followed by a meiotic or postmeiotic centromeric misdivision. Additionally, Bugge et al. [1] presented two cases with possible maternal meiosis I (MI) nondisjunction and one family with an i(18p) originating from a postmeiotic nondisjunction. Nondisjunction in paternal meiosis has rarely been identified as the cause of an autosomal trisomy. No case of additional i(18p) involving a paternal chromosome has yet been reported. In trisomy 18, 18 out of 214 cases investigated for parental origin were paternally derived, the majority of which is probably caused by postzygotic mitotic nondisjunction of paternal chromosome 18 [5–10], while allelic distribution in two of these cases pointed to paternal meiosis as the cell stage of nondisjunction [4].

Here we describe our molecular findings in a child with a supernumerary i(18p) possibly originating from a paternal MI nondisjunction followed by isochromosome formation.

The girl was examined at the age of 2 years because of muscular hypertonia, psychomotor retardation and microcephaly. Craniofacial anomalies included bitemporal hollowing, lateral displacement of inner canthi, epicanthic folds, downslanting palpebral fissures, strabismus, broad nose root, small ears, small, often open mouth, everted lower lip and high palate. The fingers were distally tapering and held in hypertonic contractures, the toes were hypoplastic. The parents were healthy and unrelated. They both had normal karyotypes. The maternal age at the time of conception was 23, the paternal age 45 years.

GTG banding analysis revealed a small metacentric chromosome (SMC) roughly consistent in size and G-banding pattern with an i(18p). After fluorescence in situ hybridization (FISH) with a DNA probe specific for the centromere of chromosome 18 (D18Z1, Appligene Oncor) a distinct hybridization signal was detected in the middle of the SMC as well as on the centromeres of the normal chromosomes 18. SMCs of the patient were isolated by chromosome microdissection. After DOP-PCR amplification of the isolated SMC a reverse hybridization onto metaphases of the patient was performed according to methods described elsewhere [2, 11]. Hybridization signals covered the whole SMC in addition to the short arms of both chromosomes 18. FISH results thus strongly suggest the SMC to be a monocentric isochromosome 18p.

In order to determine the parental origin and the mode of formation of the isochromosome 18p, we typed 5 short tandem repeat markers (STRs) from the short arm and one from the long arm of chromosome 18 (table 1). Additionally, we typed 5 STRs from chromosomes 6 and 17. DNA was extracted from venous blood samples as described by Miller et al. [12]. STR primer sequences were obtained from the Genome Database. PCR conditions and polyacrylamide gel electrophoresis as well as visualization of the amplification product by silver staining or by autoradiography were performed as described elsewhere [4]. The alleles were scored according to the proven presence of tetrasomy 18p, i.e. an 18p STR showing three alleles with the smallest allele of significantly stronger intensity would be scored as 1-1-2-3, a polymorphism showing two alleles with the bigger allele of stronger intensity would be scored as 1-2-2-2, etc.

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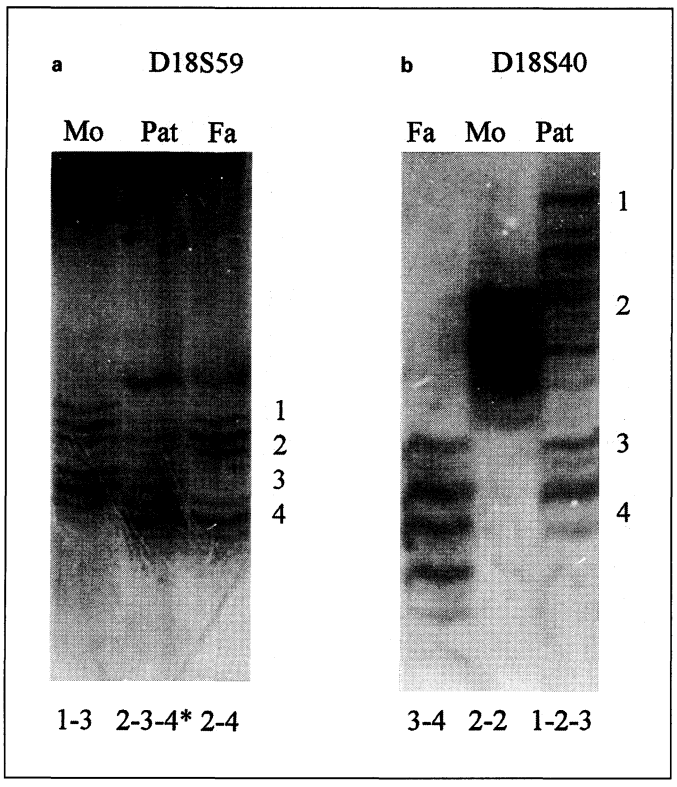

Fig. 1. Examples for STR typing in our patient. **a** Typing of D18S59 showed maintenance of paternal heterozygosity in the child. **b** PCR amplification product of the marker D18S40 showed three different alleles in the proband's DNA. Allele 2 is inherited from the mother, allele 3 from the father, but the origin of the allele 1 is hypothesized to be the result of a mutational event. (Fa = Father, Mo = mother, Pat = patient; * the allelic bands show a stronger intensity.

Table 1. Molecular analysis of parental origin of the supernumerary i(18p) in our proband

Locus	Allelic status of			Informativeness
	father	mother	child[a]	
D18S59	2-4	1-3	2-3-4-4	paternal NR
PACAP	1-2	3-4	1-1-2-3	paternal NR
D18S453	1-2	1-3	1-1-2-3	– NR
D18S37	2-2	1-2	1-2-2-2	– –
D18S40	3-4	2-2	1-1-2-3[b]	mutational event
cen				
D18S61	1-2	3-4	2-4	biparental

The order of the STRs accords with the genetic map of human chromosome 18 published in the report of the Second International Workshop of Human Chromosome 18 Mapping [18] and with the map published by Rojas et al. [14]. NR = Nonreduction of paternal heterozygosity to homozygosity; – = uninformative.

[a] The child's allelic status showed paternal alleles in higher intensities pointing to a tetrasomy.

[b] The child's allelic status can only be explained by a mutational event (see text).

By typing these polymorphisms we were able to demonstrate that the additional chromosome was paternal in origin (table 1): the distal short arm markers D18S59 (fig. 1a) and PACAP showed nonreduction of paternal heterozygosity. A third short arm marker, D18S453, revealed nonreduction of heterozygosity, but the parental origin could not be determined. The STR D18S37 was not informative. A mutation seems to have occurred in the most proximal short arm marker D18S40 (fig. 1b): the child showed a paternal as well as a maternal allele but also a third allele that did not correspond to any parental allele. We confirmed paternity by typing 5 different markers of chromosomes 6 and 17, all of which showed allele patterns consistent with paternal inheritance. Assuming a mutational event, a dinucleotide repeat expansion of more than three repeats would have affected one paternal allele. Such length variations can be caused by strand slippage during replication and have been described before. Weber and Wong [13] observed mutation rates for individual STRs ranging from 0 to 8 $\times$ 10^{-3} per locus per gamete per generation. Typing the STR D18S40 in more than 100 families we have never observed a mutation as described above.

There was no difference between the clinical findings in our child and in the two other children with maternally inherited tetrasomy 18p studied in our laboratory [unpubl. data; 2] and in that from other groups [1, 3].

Nonreduction of heterozygosity in the child's analyzed 18p loci points to nondisjunction in paternal MI, assuming that no recombination has occurred between the centromere and D18S453 which according to the map published by Rojas et al. [14] is equivalent to a maximum genetic distance of 6 cM. But it has to be taken into consideration that the mutational event in the locus D18S40 could be due to a recombination. Therefore, there would be homozygosity for very proximal segments of 18p, which would imply paternal MII nondisjunction. The typing of the 18q polymorphism D18S61 indicates that the two normal homologues 18 are of biparental origin.

Assuming maintenance of paternal heterozygosity in the whole i(18p), the isochromosome formation in our case could be explained by a centromeric misdivision in a paternal germinal premeiotic mitosis followed by an MI nondisjunction, or a paternal MI nondisjunction followed by a centromeric misdivision [6]. In both modes of formation, a nondisjunctional event took place in paternal meiosis I. So far, no nondisjunction concerning paternal MI has been reported neither for isochromosome 18p nor for trisomy 18 [1–10]. If the dinucleotide repeat expansion in D18S40 is caused by a recombination, indicating homozygosity of the proximal segment of the i(18p), nondisjunction took place in paternal meiosis II, followed by isochromosome formation. Both in trisomy 18 and supernumerary i(18p) formation maternal MII seems to be by far the most frequent cell stage of origin, while nondisjunction in paternal meiosis seems to be a rare event in the etiology of human autosomal trisomies [15–17].

Chromosome 18 seems to be unique among human autosomes in respect of a predisposition to maternal MII nondisjunction, a phenomenon which was first reported for trisomy 18 and which is now confirmed by most studies on i(18p) formation [1–3], although the findings in our case show clearly that exceptions do exist.

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