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ORIGINAL PAPER

Isochromosomes 12p and 9p: parental origin and possible mechanisms of formation

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In a recent study Bugge *et al*[1] and Kotzot *et al*[2] reported that isochromosomes 18p originate mainly from maternal meiosis II nondisjunction, followed by misdivision. In order to determine if there is a common mechanism for isochromosome formation, three cases with mosaicism for an additional isochromosome 12p and three cases with tetrasomy 9p were studied. Two probands with isochromosomes 12p and the three cases with isochromosome 9p showed 3 alleles (two different maternal alleles and one paternal allele) at several loci mapping to distal 12p and 9p, respectively. Maternal heterozygosity for distal markers was reduced to homozygosity for markers closer to the centromere in both i(12p) cases and in one i(9p) case. For one patient with isochromosome 12p, the maternal band was clearly stronger than the paternal one at some loci, but two distinct maternal alleles were never seen. For one foetus and the patient with tetrasomy 9p, distal markers showed maternal heterozygosity. All proximal markers were not informative in these two i(9p) cases. Our findings indicate common features in different autosomal isochromosomes: the origin of the isochromosomes analysed is predominantly maternal; and a common mechanism appears to underlie their formation, namely due to meiosis II nondisjunction followed by a rearrangements leading to duplication of the short and loss of the long arm.

Keywords: isochromosome 9p; isochromosome 12p; tetrasomy

Introduction

In a recent study, Bugge *et al*[1] and Kotzot *et al*[2] showed that isochromosomes 18p predominantly originate through maternal meiosis II nondisjunction, followed by a rearrangement or centromeric misdivision. The same origin had previously already been demonstrated in a case with an additional isochromosome 8p.[3] Only one exception was reported among 20 cases: a patient with an additional isochromosome 18p of paternal origin.[4] Four families with an index patient with mosaicism for an additional 12p have so far been typed for origin and mechanism of formation.[5,6] The marker was of maternal origin in three and of paternal origin in one.[5] The authors did not raise the issue of meiosis I versus meiosis II and mitosis as the stage of occurrence of nondisjunction, and the markers typed did not allow a clear distinction. Markers with three different alleles were found in two patients[5,6] while in the two others,[5] including the case of paternal origin, all markers examined showed homozygosity.

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In order to determine whether the same mechanisms are also responsible for the formation of other additional autosomal isochromosomes, we investigated three patients with additional isochromosomes 12p and three cases with additional isochromosomes 9p and their parents. Origin and mode of formation of the additional isochromosomes were investigated using microsatellite markers.

Patients and Methods

Patients 1–3 with Pallister-Killian syndrome (mosaic-tetrasomy 12p)

Findings present in all three patients included normal growth and head circumference, temporal balding, curled hair, prominent and broad forehead, inner epicanthic folds, upslanting palpebral fissures, short nose with bulbous tip, prominent upper lip with distinct philtrum, irregular position of incisors, high-arched palate, hypoplastic finger and toe nails, especially the fifth, cerebral atrophy at MRI, epilepsy and profound mental retardation.

Patient 1 is a male, presently 4 years old. Both the mother and the father were 39 years old at delivery; the couple had one previous liveborn and two spontaneous abortions. The proband was delivered at 37 weeks of gestation, birth weight was 3970 g, his length was 52 cm and the head circumference was 34 cm. Additional clinical findings include narrow palpebral fissures, exophthalmos, hypertelorism, bilateral supernumerary nipples, intestinal malrotation with volvulus, right undescended testis, hypertrichosis of the back, central amaurosis, and myelinisation defect at MRI. At age 4 the child has a mental development corresponding to less than one year.

Patient 2 is a female, presently 5 years old. The maternal and paternal ages at birth were 39 and 34 years, respectively. The couple had three previous spontaneous abortions and two liveborns. The proband was delivered at 40 weeks of gestation, at birth she weighed 3650 g and her length was 50 cm. Additional findings include prominent occiput, arched eyebrows with synophrys, broad septum of the nose, small ears, prominent lower lip, hypoplastic labia, and anal stenosis.

Patient 3 is a male, presently 5 1/2 years old. The maternal and paternal ages at birth were 30 and 27 1/2 years, respectively. There were no previous gestations. The proband was delivered at 40 weeks, his birth weight was 3580 g, his length was 50 cm, and the head circumference was 35 cm. Additional findings include prominent tongue, short and tapering fingers, and hyper- and hypopigmented areas of skin over the abdominal regions and both extremities.

Patients 4–6 with additional, isochromosome 9p (non-mosaic tetrasomy 9p)

Foetus 4 was a male. The maternal and paternal ages at expected term were 31 and 32 years, respectively. The mother had one previous induced abortion. Abnormal foetal ultrasonographic findings (growth retardation, cleft lip and palate, possible brain malformation) prompted a chorionic villus chromosome examination. The pregnancy was terminated at 17 2/7 weeks when the foetus was already spontaneously deceased *in utero*. Weight was 64 g and length was 14 cm. Abnormalities included brachycephaly, large anterior fontanelle, low-set and misshapen ears, bilateral complete cleft lip and cleft palate, persistent left and rudimentary right cava superior vein, ascites, cystic-dysplastic kidneys, and diminished mineralisation of vertebrae and pelvic bones on radiographs.

Foetus 5 was a male; maternal and paternal ages at expected term were 35 and 37 years, respectively. It was the mother's third pregnancy following two normal boys. Abnormal foetal ultrasound findings (polyhydramnios, intrauterine growth retardation, abnormal position of fingers, rockerbottom feet) prompted amniocentesis at 32 weeks of age. At 33 6/7 weeks, intra-uterine death of the foetus was detected and delivery induced on the same day. The foetus weighed 1330 g and measured 39.5 cm in length. Abnormalities included a sloping forehead, hypertelorism, downslanting palpebral fissures, left complete cleft lip and cleft palate, low-set, prominent and dysplastic ears, absence of the gallbladder, short hands and feet, a left transverse Palmas crease, hypoplasia of distal phalanges and nails, especially of the thumbs and fifth fingers, rockerbottom feet and a left pes adductus. The heart and great vessels as well as the kidneys were normal at autopsy.

Patient 6 is a female, presently 21 years old. The maternal and paternal ages at birth were 31 and 30 years, respectively. One older sister and two younger brothers are healthy and normal. During pregnancy the mother felt that foetal activity was less than in the other gestations. Delivery took place at 42 weeks, birth weight was 2700 g, length was 48 cm, and head circumference was 33.5 cm. There was mild postnatal asphyxia and hypoglycaemia. Multiple minor abnormalities were noticed. Subsequent motor and mental development was severely delayed with sitting at 10 months, first free steps at 3 1/2 years, first words at 4 years. Urine continence was never achieved. At age 21 years, height was 1.54 cm (3rd–10th percentile), weight was 54 kg (50th percentile), head circumference was 55.5 cm (90th percentile), hand length was 18 cm (50th–75th percentile), and foot length was 22.5 cm (20th percentile). She displayed the following abnormal findings: small, low-set and prominent ears (ear length 5.0 cm, 3rd percentile), a flat occiput, hypertelorism (inner canthal distance 4.5 cm, > 97th percentile), downslanting palpebral fissures, a large nose with a prominent bridge, a short and prominent upper lip, downturned corners of the mouth, an asymmetric mandible deviated to the right, hypoplastic mammals and nipples, pectus excavatum, distinct thoracic kyphosis and moderate scoliosis, a small umbilical hernia, normal pubic hair distribution, normal hands and fingers. There was myopia of about 2D. Her mental development corresponded to about 2 years of age.

Cytogenetic Investigations

GTG-banded chromosomes were investigated from lymphocyte and fibroblast cultures of patients 1–3 and 6, from amniotic fluid cell cultures of patients 4–5 and from chorionic villus cell cultures of patient 4. In all six families, GTG-banded chromosome studies were also performed from lymphocytes of both parents in order to exclude a familial rearrangement preceding isochromosome formation.

Molecular Investigations

Genomic DNA was extracted from peripheral blood and cultured skin fibroblasts (patients 1, 2, 3 and 6), muscle and umbilical cord (patient 4) and cultured amniotic fluid cells

Table 1a Results of microsatellite examination in families 1–3 with proband with additional mosaic 1(12p)

Primers	*Locus*	*Family 1*	*Family 2*	*Family 3*
D12S352	12pter-p13.2	ab,ab,bc	**abc,ac,bd**	*acc,bc,aa*
D12S94	12p13	ab,ab,aa	abc,bc,ac	aab,aa,bc
Y21	12p13	bc,bc,ab	abc,ac,ab	ab,ac,bb
D12S99	12pter-p13.2	**abc,ab,cd**	bbc,bb,ac	abb,bb,ab
CD4	12pter-p12	**abc,bc,aa**	abb,bb,ab	ab,ab,ab
D12S77	12pter-p13.2	abc,ab,bc	abc,bc,ac	ab,ab,ac
DRPLA	12p13.31	**abd,bd,ac**	abc,bc,ab	ab,ac,bd
D12S320	12p12	ab,ab,ab	aa,aa,aa	acc,cc,ab
D12S269	12p12	bc,bb,ac	abb,bb,ab	ac,cc,ab
D12S61	12p12-p11	abb,bb,aa	abb,bb,ac	bb,bb,ab
D12S345	12p11.2	*aab,ac,bc*	*aac,ab,bc*	*aad,ac,bd*
D12S82	12q	ab,ac,bc	aa,ab,aa	ab,aa,bb
D12S72	12q	cc,ac,bc	aa,ab,ab	ab,aa,bb
D12S43	12q12-24.1	nd	ab,ac,bc	nd

The alleles are given in the order: patient, mother, father. The order of the loci is from distal short arm (top) to long arm (bottom). Markers with two different maternal alleles are given in bold, markers showing reduction to homozygosity in italics, and markers mapping to the long arm of chromosome 12 and showing normal biparental inheritance are underlined. Allele designations (a to d) are arbitrary. nd = not done.
Due to mosaicism as well as the non-quantitative characteristics of PCR amplification, only a semi-quantitative analysis was possible and in most cases only the distinct alleles are indicated.

(patients 4–5) by standard methods. Microsatellite loci are listed in Table 1a and b map to the short arm of chromosome 12 in families 1–3, and to the short arm of chromosome 9 in families 4–6. Additional markers mapping to the long arms of the isochromosomes were applied in order to exclude uniparental disomy. The localisation of the markers is based on the CEPH/Genéthon and GDP maps. All primers were obtained from Research Genetics (Huntsville, U.S.). Between 200 and 500 ng of DNA were amplified with the indicated primers in a volume of 25 µl. Polymerase chain reaction (PCR) amplification was performed on a Perkin-Elmer 9600 with 32 cycles of 30 sec at 94°C, 45 sec at 55°C to 60°C for the annealing, and 1 min 20 sec at 72°C for the extension. The reaction product was mixed in an equal volume of urea loading buffer (42% urea, 0.1% xylene cyanol, 0.1% bromphenol blue, and 0.1% 0.5 M EDTA) and was loaded on to a 0.4 mm thick 6% polyacrylamide/50% urea gel. Visualisation of bands was done by silver staining of the gels.

Results

Cytogenetic Findings

In patients 1–3 with the phenotype of Pallister-Killian syndrome, chromosome examination from skin fibroblast cultures revealed an isochromosome 12p in addition to a normal karyotype. The extra chromosome was present in 94 out of 100 fibroblast metaphases from patient 1, in 56 out of 100 cells from patient 2, and in 47 out of 50 from patient 3. However, the blood lymphocyte karyotypes in patients 1, 2, and 3 were normal diploid on 100, 100 and 50 examined cells, respectively.

For patient 4 with tetrasomy 9p, the additional isochromosome was detected in all nine investigated chorionic villus cells and seven cultured amniotic fluid cells, and patient 5 revealed the additional i(9p) in 29/30 cultivated amniotic fluid cells. Breakpoints most likely were at 9q12. In patient 6, the additional isochromosome 9p was present in all 30 metaphases of a lymphocyte culture at the first examination, when the patient was 8 1/2 months old. In a recent examination at the age of 21 years the i(9p) was present in 48/50 lymphocytes.

Parental karyotypes were normal in all 6 families.

Molecular Findings

DNA analysis indicated that all six extra isochromosomes were of maternal origin (Table 1a and b). In respect of additional i(12p), in families 1 and 2, the distal 12p markers showed two maternal alleles and one paternal allele in the patients (Figure 1a, Table 1a). In

Table 1b Results of microsatellite examination in families 4–6 with proband with additional i(9p)

Primers	*Locus*	*Family 4*	*Family 5*	*Family 6*
D9S281	9p24-23	bc,bc.ab	ab.ab.ab	ab,ab,bb
D9S286	9pter-p22	**acd,ad,bc**	**acd,ac,bd**	**abc,ac,ab**
D9S144	9pter-p22	nd	nd	**abc,bc,aa**
D9S157	9p23-p22	bb,bb,ab	**abc,ac,bb**	**abc,ab,ac**
D9S171	9p21	**bcd,bd,ac**	abb,ab,bc	aa,aa,aa
D9S104	9p21	nd	nd	**abc,ac,bd**
D9S304	9p21	**acd,ad,bc**	**bcd,bd,ac**	bc,bc,ab
D9S749	9p24-p21	nd	*aac,ab,cc*	nd
D9S200	9p21-p12	abb,bb,ac	bb,bb,ab	aab,aa,bb
D9S55	9p12	abb,bb,ab	*acc,ac,ab*	abb,bb,aa
D9S43	9p21	**acd,ac,bd**	aab,aa,ab	ab,ab,ab
D9S273	9p21-q21	ab,bb,ac	ab,aa,ab	ad.ab,cd
D9S166	9p21-q21	bc,ac,ab	nd	nd
D9S175	9q13-21	ab,bc,ac	ab,bd,ac	bc,ac,bc
D9S155	9q32-33	ab,aa,bc	bc,ac,bb	ab,ab,ab
D9S53	9q22.3-31	nd	nd	ad,cd,ab

The alleles are given in the order: patient, mother, father. The order of the loci is from distal short arm (top) to long arm (bottom). Markers with two different maternal alleles are given in bold, markers showing reduction to homozygosity in italics, and markers mapping to the long arm of chromosome 9 and showing normal biparental inheritance are underlined. Allele designations (a to d) are arbitrary. nd = not done.
Due to the non-quantitative characteristics of PCR amplification, only a semi-quantitative analysis was possible and in most cases only the distinct alleles are indicated.

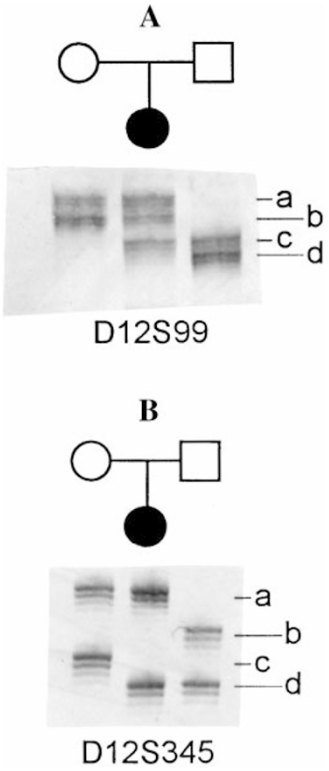

Figure 1 (a) *Results for the telomeric marker D12S99 in family 1. The patient has inherited two maternal alleles (a, b) and one parental allele (c).* **(b)** *Results for the centromeric marker D12S345 in family 3. The patient has inherited the maternal allele a presumably in triplicate and a single copy of the paternal allele d.*

the centromeric region, the two maternal alleles were reduced to homozygosity in patients 1 and 2, with only one maternal allele with stronger intensity probably representing the isochromosome and the normal maternal chromosome 12 (Table 1a). In the patient from family 3 (Figure 1b) no marker showed three alleles, but for several markers (including also the proximal one) the maternal allele showed a stronger signal than the paternal one, confirming maternal origin of the marker chromosome. Uniparental disomy of the normal chromosome 12 was excluded through demonstration of biparental inheritance of markers on 12q.

PCR analysis of DNA extracted from the tissues of foetus 4 and patient 6 with additional i(9p) showed two maternal alleles for distal 9p markers (Figure 2, Table 1b). Normal biparental inheritance of 9q markers (Table 1b) excluded maternal uniparental disomy. The findings in foetus 5 were similar to those in patients 1 and 2: at distal loci, one parental and two different maternal bands were present while there was reduction to homozygosity at a more proximal informative marker (D9S55 mapping to 9p12, see Table 1b).

Discussion

In families 1 and 2 with proband with additional i(12p), and in foetus 5 with extra isochromosome 9p, maternal heterozygosity was preserved for the telomeric markers, but maternal alleles were reduced to homozygosity in the centromeric region. Thus, the additional isochromosomes most likely resulted from a meiosis II nondisjunction of chromosome 12 and 9, respectively, in the mother, followed by meiotic or postmeiotic mitotic misdivision or recombination in the false direction at the centromere and subsequent loss of the long arms.

The proband of family 3 [+ i(12p)] showed reduction of maternal heterozygosity to homozygosity along the whole chromosome 12p suggesting a postzygotic nondisjunction event, followed by misdivision. However, meiosis II nondisjunction cannot be excluded, firstly because there might be more telomeric non-reduced regions for which no primers are available, and secondly because no recombination in the short arm might have occurred in this case.

For foetus 4 and patient 6 with i(9p) we were able to determine the maternal meiotic origin of the marker chromosome and maternal heterozygosity for all informative markers from the telomere to 9p12. Since no informative more proximal markers are available for study, it remains unclear whether the isochromosome was formed by meiosis I nondisjunction without previous recombination or by meiosis II nondisjunction

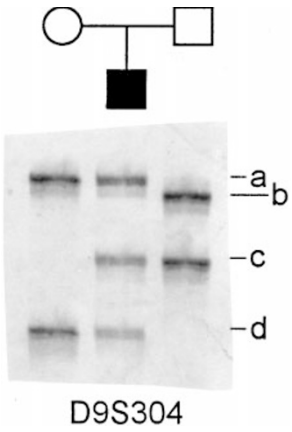

Figure 2 *Result for marker D9S304 mapping to 9p21 for foetus 4 with i(9p). The patient has inherited the two maternal alleles a and d, and the paternal allele c.*

with a recombination proximal to D9S43, the most proximal informative marker, each event being followed by a recombination leading to duplication of the short and loss of the long arm. For the different modes of origin of additional isochromosomes see also Kotzot *et al*[2] and an illustrative diagram in Bugge *et al*.[1]

The results of similar investigations in families with a proband with additional isochromosome 18p,[1,2] the single result on a case with a supernumerary isochromosome 8p,[3] and the cases of this report seem to suggest a common parental origin. A maternal origin is found in the overwhelming majority of cases with additional autosomal isochromosomes. The most frequent mechanism of formation would involve meiosis II nondisjunction immediately followed by rearrangements which lead to duplication of the short and loss of the long arm.

The high incidence of meiosis II nondisjunction detected in these cases is significant (in contrast to the predominance of meiosis I nondisjunction in most chromosomes except for chromosome 18[7]) and suggests an association between second meiotic disjunctions and the as yet unknown mechanism by which isochromosomes are secondarily formed. The advanced mean maternal age at delivery known from previous reported cases with additional i(12p) and i(18p) as well as in our families is a further indication that meiotic nondisjunction may be the first step in the formation of isochromosomes. However, more data are required to confirm these conclusions and find out whether and if so how frequent other mechanisms occur. Furthermore, it would be interesting to examine whether the same mechanisms could also underlie the formation of other supernumerary isochromosomes or isodicentric chromosomes.

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Note added in proof:

A further case with an additional i(12p) was analysed after revision of the manuscript. The foetus was a female, the pregnancy was terminated at 20 weeks of gestation. The 33 year old mother had 2 previous pregnancies which resulted in two healthy children. The foetus weighed 250 g (within normal limits of gestational age) and presented with hypertelorism, depressed nasal bridge, upturned nares and prominent forehead. Further clinical findings included VSD, lung hypoplasia and single umbilical artery. The patient's karyotype was determined prenatally (amniotic fluid cells) and postmortem (fibroblast cells). In the first examination the karyotype was 47, XX, +i(12)(p10) [16]/46, XX, [3] (confirmed by FISH), and in the second 47, XX, +i(12)(p10) [21]/46, XX, [29]. The molecular results obtained for this patient are essentially similar to those for patients 1 and 2, namely two different maternal alleles and 1 paternal allele at the distal positions (pter-p12) and reduction to homozygosity of the maternal alleles at proximal positions (from p12). The following markers showed 3 different alleles: D12S1685, which is also informative as to the maternal origin, D12S99 and D12S269. Marker D12S61 showed reduction to homozygosity. Further 4 markers mapping to the short arm were tested but were not informative. Regarding the long arm of chromosome 12, D12S82 and D12S43 show normal biparental inheritance.

References

1 Bugge M, Blennow E, Friedrich U *et al*: Tetrasomy 18p *de novo*: parental origin and different mechanisms of formation. *Eur J Hum Genet* 1996; **4**: 160–167.
2 Kotzot D, Bundscherer G, Bernasconi F *et al*: Isochromosome 18p results from maternal meiosis II nondisjunction. *Eur J Hum Genet* 1996; **4**: 168–174.
3 Fisher AM, Barber JCK, Crolla JA, James RS, Lestas AN, Jennings I, Dennis NR: Mosaic tetrasomy 8p: molecular cytogenetic confirmation and measurement of gluthathione reductase and tissue plasminogen activator levels. *Am J Med Genet* 1993; **47**: 100–105.
4 Eggermann T, Engels H, Apacik C, Moskalonek B, Müller-Navia J, Schwanitz G, Stengel-Rutkowski S: Tetrasomy 18p caused by paternal meiotic nondisjunction. *Eur J Hum Genet* 1997; **5**: 175–177.
5 Turleau C, Simon-Bouy B, Austruy E *et al*: Parental origin and mechanisms of formation of three cases of 12p tetrasomy. *Clin Genet* 1996; **50**: 41–46.
6 Cormier-Daire V, Le Merrer M, Gigarel N *et al*: Prezygotic origin of the isochromosome 12p in Pallister-Killian syndrome. *Am J Med Genet* 1997; **69**: 166–168.
7 Fisher JM, Harvey JF, Morton NE, Jacobs PA: Trisomy 18: studies of the parents and cell division of origin and the effect of aberrant recombination on nondisjunction. *Am J Hum Genet* 1995; **56**: 669–675.