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First Patient With Trisomy 21 Accompanied by an Additional der(4)(:p11 → q11:) Plus Partial Uniparental Disomy 4p15-16

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We report on a rare additional numerical chromosomal aberration in a child with Down syndrome due to free trisomy 21. The karyotype showed 48,XY,+21,+mar after GTG banding, with the marker present in 80% of cells. The supernumerary marker chromosome (SMC) was as small as approximately one-third of 18p, and with the recently developed centromere-specific multi-color fluorescence in situ hybridization (cenM-FISH) technique, it was shown that the SMC was a derivative chromosome 4. The SMC was not specifically stained by arm-specific probes for chromosome 4; thus, it has been described as der(4)(:p11 → q11:). Microsatellite analysis resulted in a partial maternal uniparental isodisomy (UPD) for chromosome 4p15–16 and a maternal origin for two chromosomes 21. Until now only two similar cases have been described in the literature, but without clarifying the origin of the SMC and without looking for an additional UPD. This is the only reported case of a UPD 4p in a liveborn child.

KEY WORDS: Down syndrome; supernumerary marker chromosome; centromere-specific multicolor-FISH; uniparental disomy

INTRODUCTION

The prevalence of Down syndrome is approximately 1.66 per 1,000 cases, and in the overwhelming majority of cases, the reason for the condition is a free-standing trisomy 21 [Stoll et al., 1998]. The most frequent additional numerical chromosomal aberrations in addition to trisomy 21 are aneuploidies of the gonosomes, leading to karyotypes like 48,XXY,+21 [Lorda-Sanchez et al., 1991], 48,XXX,+21 [Park et al., 1995], or 48,XYY,+21 [Stevens et al., 1995]. Small supernumerary marker chromosomes (SMC), on the other hand, are found in 0.01–0.05% of liveborn infants [Buckton et al., 1980]. Only two cases with a combination of free trisomy 21 plus an additional small SMC have been described up to now [Osztovics et al., 1982; Sachs et al., 1987]. Here, we describe the first detailed characterization of a very small SMC, as small as approximately one-third of 18p, identified by GTG-banding analysis in a newborn boy presenting typical signs of Down syndrome. The karyotype showed mosaicism for 48,XY,+21,+mar. Centromere-specific multicolor fluorescence in situ hybridization (cenM-FISH), centromere 4-specific interphase FISH, two-color FISH using arm-specific probes for chromosome 4, and microsatellite analyses have been used to clarify the origin of this minute SMC, to extrapolate its clinical significance, and to exclude UPD.

MATERIAL AND METHODS

Clinical Report

The boy was the second child of healthy unrelated parents; the mother was 30 years old and the father was 35 years old at the time of his birth. The elder sib was healthy. At birth (cesarean section performed due to placenta insufficiency) after 36 weeks of pregnancy, his length (44 cm) and weight (2,180 g) were below the 10th centile. At the age of two months, his weight was 3,340 g, which was at the 10th centile. Clinical findings were in complete concordance with Down syndrome, i.e., hypermotility, single transverse crease, mild webbed neck, and typical facial dysmorphisms (brachycephaly, upslanting palpebral fissures, small nose with flat nasal root).

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TABLE I. UPD Analysis

Marker	Mother (m)	Father (f)	Child (c)	Origin of alleles
4p				
D4S2366	bc	aa	bb	mat-iso
D4S403	ab	ab	ab	n.i.
GATA145E01	aa	bb	aa	mat.
D4S2633	cc	ab	ac	n
D4S2639	cc	ab	ac	n
Centromere 4				
D4S1627	ab	ac	bc	n
4q				
D4S2367	ab	bb	bb	n.i.
D4S2361	ab	aa	aa	n.i.
D4S2623	bb	aa	ab	n
D4S2394	bc	ac	ac	n.i.
D4S1625	ab	aa	aa	n.i.
D4S2431	cd	ab	ac	n
D4S1652	ac	ab	ac	n.i.
21q				
D21S1432	ab	b	b	n.i.
D21S1270	bc	ab	ac	i
S21S1446	ab	b	ab	n.i.

Results of microsatellite analysis for chromosome 4 and 21 in the child and his parents. n.i., noninformative; n, normal; mat-iso, maternal isodisomy; mat, maternal alleles only.

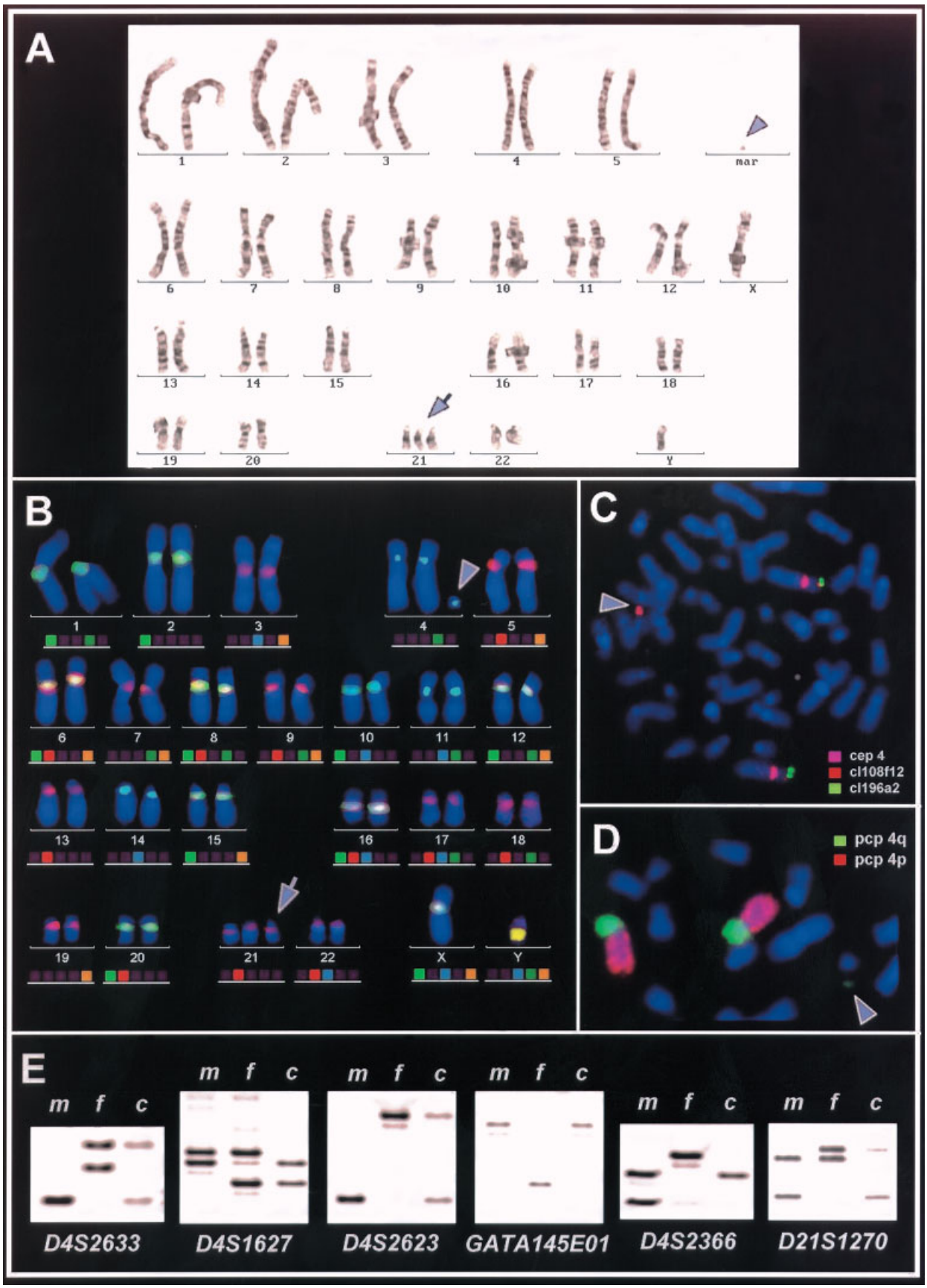
Cytogenetics and Molecular Cytogenetics

Cytogenetic and molecular cytogenetic studies were performed on chromosomes derived from peripheral blood. Chromosome preparations and GTG-banding were performed according to standard techniques [Verma and Babu,1989]. FISH, including RNase- and pepsin-pretreatment, denaturation of the slides, and addition of the probe to the sample, were performed according to standard protocols [Liehr et al., 1995] for the Wolf-Hirschhorn-syndrome region probes cl108f12 and cl196a2 [Wright et al., 1997]. CenM-FISH was performed as described elsewhere [Nietzel et al., 2001], and the results were verified using single-color FISH with a commercially available centromeric probe for chromosome 4 (Vysis). The arm-specific probes for chromosome 4 were microdissection derived and amplified, labeled, and hybridized according to Chudoba et al. [1996]. The results were evaluated on a fluorescence microscope equipped with a charged coupled device (CCD)-camera and an image analysis system (MetaSystems, Altlussheim, Germany).

Microsatellite Analysis

Genomic DNA was isolated from peripheral lymphocytes. All reverse primers were labeled with an infrared fluorescent label (IRD800; MWG-Biotech, Germany). Polymerase chain reaction (PCR) was performed in 12.5 μl volumes containing 50 ng DNA, 150 μM dNTPs, 1.5 mM $MgCl_2$, 0.2 mM forward and reverse primer, 1 × PCR buffer (Eurogentec, France), and 0.5 units Taq polymerase (Eurogentec, France). The loci used for PCR are listed in Table I (all sequence information from Cooperative Human Linkage Center (CHLC); http://gai.nci.nih.gov/CHLC/). Amplification conditions were a three-min initial denaturation at 95°C, followed by 25 cycles with 45 sec at 95°C, 45 sec at 55°C, and 45 sec at 72°C. Aliquots of the PCR products were mixed with an equal amount of formamide loading buffer. Denatured samples were loaded on a 6% denaturing polyacrylamide gel and were electrophoresed on a Licor DNA 4000 sequencer, where labeled products were detected with an infrared laser diode.

Fig. 1. (*Overleaf.*) Images were captured on a Zeiss Axioplan microscope (Zeiss Jena, Germany) with the IKAROS and ISIS digital FISH imaging system (MetaSystems, Altlussheim, Germany) using a XC77 CCD camera with on-chip integration (Sony). The SMC and the free trisomy 21 are marked throughout the figure by an arrowhead and an arrow, respectively. **A:** GTG-banding results of the boy showing clinical signs of Down syndrome. Karyotype: 48,XY,+21,+mar. **B:** CenM-FISH results of the presented case: the trisomy 21 is confirmed and the SMC is identified as a der(4). **C:** Result of a three-color FISH experiment hybridizing simultaneously commercially available centromeric probe for chromosome 4 (Vysis, SpectrumOrange), and two probes specific for the Wolf-Hirschhorn-syndrome (WHS) region (cl108f12, TexasRed; cl196a2, SpectrumGreen; probes are specified in [Wright et al., 1997]). The centromeric probe and the WHS-specific probes demonstrated that no deletion was present in 4p16.3, confirming the cenM-FISH result. **D:** Arm-specific probes for chromosome 4 (pcp4p and pcp4q) stained the two chromosomes 4 as expected, and gave only very weak signals on the SMC. For interpretation of the result see text. **E:** Results of the microsatellite analysis to exclude UPD of the two inconspicuous chromosomes 4. The five depicted microsatellite markers represent the informative situation. In D4S2633, D4S2623, and D4S1627, the child (c) has an allele of the father (f) and of the mother (m). The markers D4S2366 and GATA145E01 showed a maternal isodisomy (also see Table I). Additionally, the result of the microsatellite analysis for the determination of the origin of the third chromosome 21 is shown (also see Table I). The marker D21S1270 was informative and showed that one allele originated from the father (allele a) and one from the mother (allele c). However, the specific band for allele c is twice as intense as that of allele a in the child. Thus, it can be concluded that two copies of the same maternal chromosome 21 are present in addition to the paternal chromosome 21.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cl108f12
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pcp 4q
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E
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m f c
m f c
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D4S2633
D4S1627
D4S2623
GATA145E01
D4S2366
D21S1270

RESULTS

In a newborn boy presenting with typical clinical signs of Down syndrome, cytogenetic analysis revealed a karyotype 48,XY,+21,+mar (Fig. 1A). The small marker was present in 28 of 35 (80%) analyzed metaphase spreads. It could not be detected in 20 metaphase spreads analyzed in each of the parents.

CenM-FISH applied on metaphases of the child confirmed that a trisomy 21 was present, and moreover characterized the SMC as a derivative chromosome 4, which was completely stained by the centromere 4-specific probe (Fig. 1B). FISH with a commercially available alphoid probe for chromosome 4 (Vysis) confirmed the cenM-FISH results (Fig. 1C). These results showed that the SMC was present in 250 of 300 interphase nuclei (83.3%). Neither the father nor the mother showed nuclei with three chromosome 4-specific signals among 250 nuclei analyzed in each parent. Arm-specific probes for chromosome 4 stained the two chromosomes 4 as expected, and gave only very weak signals on the SMC. As visible in Figure 1D, the centromeric regions of normal chromosomes 4, although blocked by unlabeled COT1-DNA, are completely painted, especially by the green probe for chromosome 4p. Thus, we interpret the weak green signal on the SMC as staining of the centromeric region and describe it as a der(4)(:p11 → q11:).

For UPD analysis of chromosome 4, 13 highly polymorphic microsatellite markers for chromosome 4 were examined in this family. Results of this testing are presented in Table I, where the markers are listed in the most likely cytogenetic or physical order (Genome Database Mapview 2.4; http://www.gdb.org/gdb/). In two loci (D4S2366, GATA145E01), a maternal uniparental isodisomy (iUPD) could be detected (Table I; Fig. 1E). Five markers showed a normal situation; the remaining six loci resulted in a noninformative pattern. To exclude a microdeletion as the basis of the iUPD, FISH with locus-specific probes for the Wolf-Hirschhorn-syndrome region (see Dufke et al. [2000]) was performed. However, both inconspicuous chromosomes 4 presented with the expected one signal on each chromatid for the probes cl108f12 and cl196a2 (Fig. 1C). Additionally, the origin of the additional chromosome 21 was determined by microsatellite analysis. Only one of three markers used was informative (for details see Table I and Fig. 1E). A maternal origin of two chromosomes 21 could be detected.

DISCUSSION

Here, we describe the first case with a maternally derived trisomy 21 plus an additional partial iUPD 4p15–16.

The iUPD 4p15–16 was recognized after the characterization of a postnatally detected very small SMC. It was present in mosaic status and identified by GTG-banding analysis as being as small as approximately one-third of 18p. A detailed characterization of the SMC was performed using the recently described cenM-FISH technique, and it was determined that it consisted of chromosome 4 centromeric material. Hints that the SMC could be a ring chromosome, which would have been size variation of one or two centromeric signals on the marker, were not found in GTG-banding or in FISH analysis.

In this case, the small SMC was present in 80% of the analyzed GTG-banded metaphase plates and in 83.3% of interphase nuclei. The possibility that the present case might be a familial one has been excluded by interphase FISH with a chromosome 4-specific alpha satellite probe (three signals per interphase nucleus have never been detected). Moreover, GTG-banding analysis of the parents in 20 metaphase spreads each revealed no numerical or structural aberrations.

Microsatellite analysis uncovered a maternal origin for two of the three chromosomes 21. This finding was the more likely one, as nondisjunctional errors are in 79.24% maternal and in only 20.76% paternal chromosomes [Jyothy et al., 2001]. Additionally, a maternal iUPD for chromosome 4 was detected in two of the seven informative loci. To our knowledge, this proband represents the third case of a maternal UPD for chromosome 4. However, no liveborn child has been described with UPD in 4p15–16 up to now. Kuchinka et al. [2001] reported a complete UPD 4 in one case with intrauterine fetal death at 30 weeks, and Yang et al. [1999] described a case with partial UPD 4q22–24. No case for maternal UPD for chromosome 4 combined with a maternally derived trisomy 21 has been described before. Maternal isodisomy was found in locus D4S2366. For marker GATA145E01, mother and child were homozygous for the same allele, so an isodisomy is also possible. To exclude that iUPD was due to a microdeletion in 4p15–16, two locus-specific probes for the Wolf-Hirschhorn-syndrome region (cl108f12 and cl196a2) were applied. No hints of a microdeletion of this region were detected either by FISH or on the two inconspicuous looking chromosomes 4. Thus, in this case, phenotypic changes due to maternal UPD could be ameliorated by a trisomic rescue. The partial isodisomy in 4p might be due to a nondisjunction in maternal meiosis II and a postzygotic recombination event. The significance of UPD studies has previously been shown [Chudoba et al., 1999; Starke et al., 1999]. However, imprinting of the region 4p15–16 seems to be unlikely, as the described child shows exclusively typical Down syndrome. Nevertheless, the possibility that potential findings of a iUPD 4p15–16 are obscured by the trisomy 21 cannot be excluded.

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REFERENCES

Buckton KE, O'Riordan ML, Ratcliffe S, Slight J, Mitchell M, McBeath S, Keay AJ, Barr D, Short M. 1980. A G-band study of chromosomes in liveborn infants. Ann Hum Genet 43:227–239.

Chudoba I, Rubtsov N, Senger G, Junker K, Bleck C, Claussen U. 1996. Improved detection of chromosome 16 rearrangements in acute myeloid

leukemias using 16p and 16q specific microdissection DNA libraries. Oncol Rep 3:829–832.

Chudoba I, Franke Y, Senger G, Sauerbrei G, Demuth S, Beensen V, Neumann A, Hansmann I, Claussen U. 1999. Maternal UPD 20 in a hyperactive child with severe growth retardation. Eur J Hum Genet 7:533–540.

Dufke A, Seidel J, Schöning M, Döbler-Neumann M, Kelbova C, Liehr T, Beensen V, Backsch C, Klein-Vogler U, Enders H. 2000. Microdeletion 4p16.3 in three unrelated patients with Wolf-Hirschhorn-syndrome. Cytogenet Cell Genet 91:81–84.

Jyothy A, Kumar KS, Mallikarjuna GN, Babu Rao V, Uma Devi B, Sujatha M, Reddy PP. 2001. Parental age and the origin of extra chromosome 21 in Down syndrome. J Hum Genet 46:347–350.

Kuchinka BD, Barrett IJ, Moya G, Sanchez JM, Langlois S, Yong SL, Kalousek DK, Robinson WP. 2001. Two cases of confined placental mosaicism for chromosome 4, including one with maternal uniparental disomy. Prenat Diagn 21:36–39.

Liehr T, Thoma K, Kammler K, Gehring C, Ekici A, Bathke KD, Grehl H, Rautenstrauss B. 1995. Direct preparation of uncultured EDTA-treated or heparinized blood for interphase FISH analysis. Appl Cytogenet 21:185–188.

Lorda-Sanchez I, Petersen MB, Binkert F, Maechler M, Schmid W, Adelsberger PA, Antonarakis SE, Schinzel A. 1991. A 48,XXY,+21 Down syndrome patient with additional paternal X and maternal 21. Hum Genet 87:54–56.

Nietzel A, Rocchi M, Starke H, Heller A, Fiedler W, Wlodarska I, Loncarevic IF, Beensen V, Claussen U, Liehr T. 2001. A new multicolor-FISH approach for the characterization of marker chromosomes: centromere-specific multicolor-FISH (cenM-FISH). Hum Genet 108:199–204.

Osztovics M, Toth S, Wilhelm O. 1982. Unusual chromosome aberrations in 3 children with Down syndrome. Acta Paediatr Acad Sci Hung 23:283–289.

Park VM, Bravo RR, Shulman LP. 1995. Double non-disjunction in maternal meiosis II giving rise to a fetus with 48,XXX,+21. J Med Genet 32:650–653.

Sachs ES, Van Hemel JO, Den Hollander JC, Jahoda MG. 1987. Marker chromosomes in a series of 10,000 prenatal diagnoses. Cytogenetic and follow-up studies. Prenat Diagn 7:81–89.

Starke H, Schreyer I, Kähler C, Fiedler W, Beensen V, Heller A, Nietzel A, Claussen U, Liehr T. 1999. Molecular cytogenetic characterization of a prenatally detected supernumerary minute marker chromosome 8. Prenat Diagn 19:1169–1174.

Stevens J, Lin A, Gettig E, Filkins K, McPherson E. 1995. Another case of prenatally diagnosed 48,XYY,+21. Am J Med Genet 55:509–511.

Stoll C, Alembik Y, Dott B, Roth MP. 1998. Study of Down syndrome in 238,942 consecutive births. Ann Genet 41:44–51.

Verma RS, Babu A. 1989. Human chromosomes: principles and techniques, 2nd edition. New York: Pergamon Press. p 72–86.

Wright TJ, Ricke DO, Denison K, Abmayr S, Cotter PD, Hirschhorn K, Keinanen M, McDonald-McGinn D, Somer M, Spinner N, Yang-Feng T, Zackai E, Altherr MR. 1997. A transcript map of the newly defined 165 kb Wolf-Hirschhorn syndrome critical region. Hum Mol Genet 6:317–324.

Yang XP, Inazu A, Yagi K, Kajinami K, Koizumi J, Mabuchi H. 1999. Abetalipoproteinemia caused by maternal isodisomy of chromosome 4q containing an intron 9 splice acceptor mutation in the microsomal triglyceride transfer protein gene. Arterioscler Thromb Vasc Biol 19:1950–1955.