PRENATAL DIAGNOSIS, VOL. 15: 1155–1159 (1995)

SHORT COMMUNICATION

RENATAL DIAGNOSIS OF MOSAIC TETRASOMY 12p/TRISOMY 12p BY FLUORESCENT *IN SITU* HYBRIDIZATION IN AMNIOTIC FLUID CELLS: A CASE REPORT OF PALLISTER–KILLIAN SYNDROME

FRANS J. LOS*, DIANE VAN OPSTAL*, MARTIN P. SCHOL*, JOHANNES L. J. GAILLARD†, HELEN BRANDENBURG‡, ANS M. W. VAN DEN OUWELAND* AND PETER A. IN 'T VELD*

*Department of Clinical Genetics, University Hospital Dijkzigt, Erasmus University, Rotterdam, The Netherlands; †Department of Pathology, University Hospital Dijkzigt, Erasmus University, Rotterdam, The Netherlands; ‡Department of Obstetrics and Gynaecology, University Hospital Dijkzigt, Erasmus University, Rotterdam, The Netherlands

Received 30 January 1995
Revised 24 May 1995
Accepted 18 June 1995

SUMMARY

A prenatally detected case of a rare mosaic tetrasomy 12p/trisomy 12p is reported, presenting as the well-known accessory isochromosome 12p and a supernumerary single 12p marker in 17/24 and 6/24 clones of cultured amniotic fluid cells, respectively. The chromosomal nature of both marker chromosomes was investigated in cultured amniotic fluid cells by fluorescent *in situ* hybridization with various probes: the 12-centromeric probes pα12H8 and D12Z3, a whole chromosome 12 paint, and the chromosome 12p-specific paint M28. DNA analysis revealed a maternal origin of the extra 12p material. After counselling, the parents requested termination of pregnancy. Inspection and autopsy of the fetus revealed many of the dysmorphisms and internal structural abnormalities of the Pallister–Killian syndrome.

KEY WORDS: tetrasomy 12p; trisomy 12p; mosaicism; amniotic fluid cells; Pallister–Killian syndrome

INTRODUCTION

Prenatal detection of a supernumerary isochromosome 12p [i(12p)] has been reported several times; in most instances, it concerned a mosaic tetrasomy/disomy 12p (Shivashankar *et al.*, 1988; Sharland *et al.*, 1991; Bernert *et al.*, 1992; Wilson *et al.*, 1994). Mosaic tetrasomy/disomy 12p (or non-mosaic tetrasomy 12p) causes the Pallister–Killian syndrome (Pallister *et al.*, 1977; Schinzel, 1991). To our knowledge, only a single case of mosaic tetrasomy/trisomy/disomy 12p has been reported (Reynolds *et al.*, 1987). We describe here the prenatal detection in amniotic fluid cells of a mosaic tetrasomy/trisomy/disomy 12p investigated with conventional cytogenetic techniques and fluorescent *in situ* hybridization (FISH). In addition, DNA investigations on the parent in whom the extra chromosomal 12p material originated and a clinical description of the fetus are presented.

Addressee for correspondence: Frans J. Los, MD, PhD, Department of Clinical Genetics, University Hospital Dijkzigt, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

CCC 0197–3851/95/121155–05

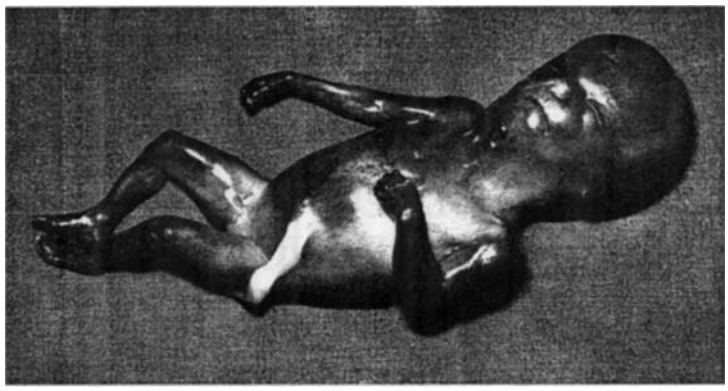

Fig. 1—The fetus at 17 weeks of gestational age displaying dysmorphic features of the Pallister–Killian syndrome

CASE REPORT

Clinical description

A 37-year-old pregnant woman (G7, P5, Ab1) visited our department for prenatal diagnosis because of advanced maternal age. Her family history revealed an autosomal dominant postaxial polydactyly, also present in two sons. Her husband's family history was unremarkable. At ultrasound investigation, a 15-week-old fetus showing a septated cystic hygroma with a thickness of 7 mm was seen. Amniocentesis was performed immediately. The parents had already been informed about a substantial risk for chromosomal abnormalities in the fetus in view of the cystic hygroma. After the finding of two different marker chromosomes, the parents requested termination of the pregnancy. At 17 weeks of gestation, labour was induced by intravenously administered prostaglandin. A female fetus of 155 g (within mean ± 1 SD; Chambers *et al.*, 1993) was delivered with multiple dysmorphisms (Fig. 1): brachycephaly, a high and broad forehead, a broad nasal bridge and hypertelorism, a very small nose with anteverted nares, a flat and long philtrum, low-set and very small ears, hygroma colli, rhizomelic shortened limbs, and postaxial polydactyly. Accessory nipples were not seen. Autopsy showed various additional internal malformations: cardiac dextro-isomerism, hypoplasia of the right ventricle and truncus pulmonalis, pulmonal valvular atresia, and a microcystic renal dysplasia. No other signs of the asplenia syndrome such as asplenia, a trilobed left lung, or failure of bowel rotation were encountered. No diaphragmatic hernia was present and the macroscopic brain obduction was normal. The dysmorphisms and internal abnormalities fitted the diagnosis of Pallister–Killian syndrome in the fetus.

Cytogenetic studies

Amniotic fluid cells were cultured according to standard techniques by the *in situ* method on glass coverslips. Trypsin–Giemsa staining was used routinely and supplemented by DA-DAPI and NOR staining because of the presence of two different marker (mar) chromosomes (Sachs *et al.*, 1987). The karyotype was 46,XX/47,XX,+mar 1/47,XX, +mar 2 in 1/17/6 clones of cultured amniotic fluid cells and in 4/18/10 subsequently cultured fetal fibroblasts. The DA-DAPI-negative and NOR-negative marker chromosomes 1 and 2, presumed to be i(12p) and 12p, respectively, are shown in Fig. 2.

Fluorescent in situ *hybridization studies (FISH)*

FISH was performed on unstained slides of cultured amniotic fluid cells which were pretreated before hybridization as described earlier (Van

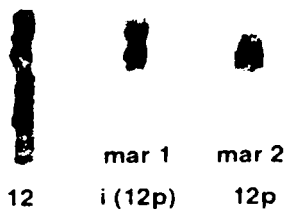

Fig. 2—Partial karyotype of cultured amniotic fluid cells (Trypsin–Giemsa staining) showing a normal chromosome 12 and both marker chromosomes

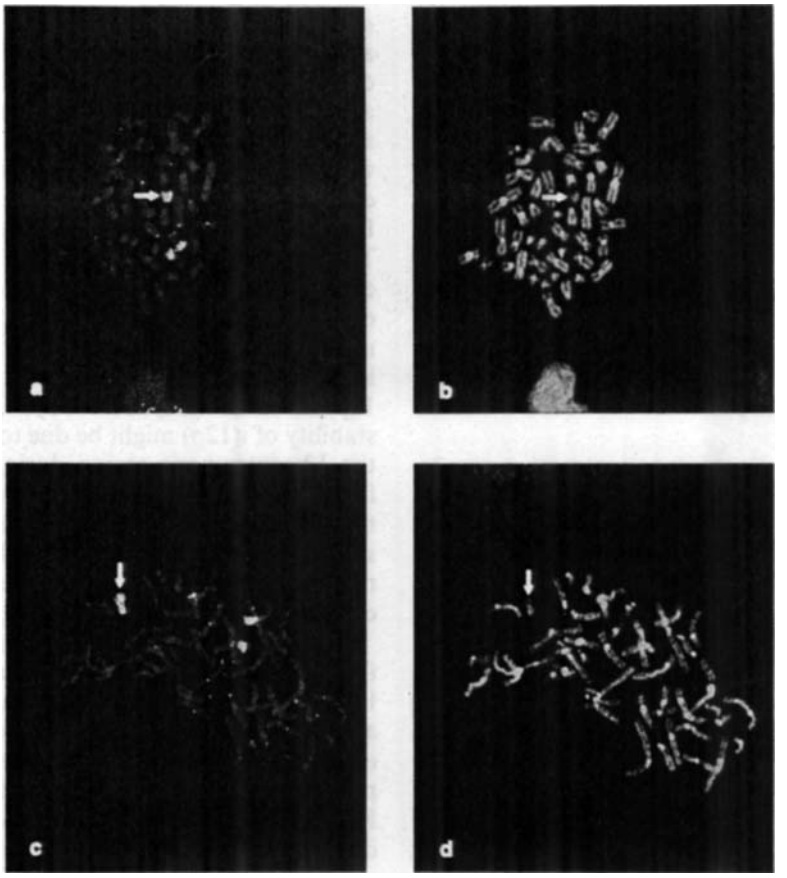

Fig. 3—FISH signals on normal chromosomes 12p and on marker chromosomes (arrow) after hybridization with M28 to amniotic fluid cell metaphases. (a) Single 12p marker and (c) i(12p) marker. (b and d) DAPI-counterstained metaphases (a) and (c), respectively

Opstal *et al.*, 1993). Our studies included the following probes: pα12H8 (D12Z1) (Looijenga *et al.*, 1990) and D12Z3 (Oncor, Gaithersburg, MD, U.S.A.), which both detect chromosome 12-specific alphoid DNA sequences; a whole chromosome 12 paint (Cambio Ltd., Cambridge, U.K.); and DNA from a mouse–human hybrid cell line, M28, containing i(12p) as the sole human chromosome (Zhang *et al.*, 1989). Pα12H8 and M28-hybrid DNA were labelled with biotin-11-dUTP by nick translation with the BioNick system (Gibco BRL, Gaithersburg, MD, U.S.A.) and hybridization took place overnight at 37°C. Immunocytochemical detection was done as described previously (Van Opstal *et al.*, 1993). FISH with the whole chromosome 12 paint and D12Z3 was performed according to the procedures recommended by the manufacturer. Slides were examined under a Leitz aristoplan fluorescence microscope and images were captured by the Genetiscan Probe Master system (Perceptive Scientific Instruments Ltd., Chester, U.K.). Hybridization with pα12H8 and D12Z3 yielded strong signals at the centromeres of the normal chromosomes 12, but no detectable signals at those of either of the marker chromosomes. Also the chromosomes 12 of both parents showed strong signals with pα12H8 and D12Z3. Hybridization with the whole chromosome 12 paint and M28 hybrid DNA resulted in fluorescent staining of both marker chromosomes, indicating a 12p origin (Fig. 3).

DNA studies

To assess the parental origin of the i(12p) and single 12p markers in the fetus, we tested a number

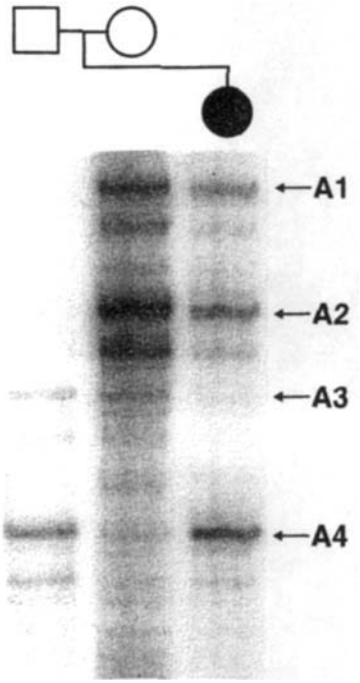

Fig. 4—PCR analysis of the polymorphic trinucleotide CAG repeats in the DRPLA gene, showing two maternal alleles (A_1 and A_2) and one paternal allele (A_4) in fetal cells

of polymorphic repeat markers by performing PCR analysis of DNA of the parents and DNA isolated from cultured fetal fibroblasts. The PCR products, obtained with the CTG-B17 primers, of the trinucleotide CAG repeats in the dentatorubral and pallidoluysian atrophy (DRPLA) gene located on chromosome 12p (Nagafuchi *et al.*, 1994) showed an informative pattern (Fig. 4). The fetus had inherited both maternal alleles A_1 and A_2 and only the paternal allele A_4. Therefore, both marker chromosomes were of maternal origin.

DISCUSSION

FISH with M28-hybrid DNA enabled the unequivocal identification of both marker chromosomes in cultured amniotic fluid cells as single 12p and i(12p), respectively. Confirmatory studies in cultured as well as uncultured skin fibroblasts or blood lymphocytes have earlier shown the usefulness of FISH in identifying i(12p) markers (Shivashankar *et al.*, 1988; Speleman *et al.*, 1991; McLean *et al.*, 1992; Blancato *et al.*, 1992; Wilson *et al.*, 1994). However, we could not detect signals at the centromeric regions of either of the marker chromosomes after FISH with the probes pa12H8 and D12Z3, although the normal fetal chromosomes 12 as well as those of both parents were positive. This phenomenon has also been encountered in fibroblasts of a patient with the Pallister–Killian syndrome (Speleman *et al.*, 1991). Therefore, caution is required when using the centromeric probes pa12H8 and D12Z3 for the detection or identification of i(12p). The single 12p marker probably originated from the unstable i(12p) marker with subsequent loss of the other 12p material (Reynolds *et al.*, 1987). The instability of i(12p) might be due to the alterations in the 12-centromeric region during isochromosome formation in our case. DNA investigation showed that the extra 12p material of the marker chromosomes was of maternal origin. However, no statement can be made about the exact maternal chromosome(s) 12 involved.

The dysmorphisms and structural internal malformations encountered in our case fitted the Pallister–Killian syndrome in the fetus (Steinbach and Rehder, 1987; Bresson *et al.*, 1991; McLean *et al.*, 1992; Bernert *et al.*, 1992). However, the polydactyly could either be part of the Pallister–Killian syndrome or the expression of autosomal dominant heredity in the mother's family. Dysmorphisms of the (partial) trisomy 12p syndrome (Bijlsma *et al.*, 1978; Stengel-Rutkowski *et al.*, 1981; Rivera *et al.*, 1987) strongly resemble those of the (mosaic) tetrasomy 12p syndrome (Pallister *et al.*, 1977; Steinbach and Rehder, 1987; Reynolds *et al.*, 1987), but structural malformations such as arrhinencephaly, diaphragmatic hernia, malrotation of the intestine, renal dysplasia, and rhizomelic shortened limbs have only been reported in association with (mosaic) tetrasomy 12p (Schinzel, 1991) and not with trisomy 12p (Stengel-Rutkowski *et al.*, 1981). Malformations belonging to the asplenia/polysplenia syndrome such as trilobed left lung (Reynolds *et al.*, 1987), accessory spleen (Warburton *et al.*, 1987; McLean *et al.*, 1992), malrotation of the intestine (Steinbach and Rehder, 1987; Warburton *et al.*, 1987), and accessory left lobe of the liver (Narahara *et al.*, 1988) have been mentioned in cases of tetrasomy 12p. Our case with dextroisomerism of the heart represents another malformation of this syndrome. The short arm of chromosome 12 seems to be involved in the development of lateralization of the internal organs.

ACKNOWLEDGEMENTS

We wish to express our gratitude to Professor A. M. Hagemeijer-Hausman and Professor A. Geurts van Kessel for providing us with the M28 DNA and to Mr A. Braat for excellent support in photography.

REFERENCES

Bernert, J., Bartels, I., Gatz, G., Hansmann, I. Heyat, M., Niedmann, P.D., Rehder, H., Waldenmaier, C., Zoll, B. (1992). Prenatal diagnosis of the Pallister–Killian mosaic aneuploidy syndrome by CVS, *Am. J. Med. Genet.*, **42,** 747–750.

Bijlsma, J.B., de France, H.F., Bleeker-Wagemakers, L.M., Dijkstra, P.F. (1978). Double translocation t(7;12),t(2;6) heterozygosity in one family. A contribution to the trisomy 12p syndrome, *Hum. Genet.*, **40,** 135–147.

Blancato, J.K., Hunt, M., George, J., Katz, J., Meck, J.M. (1992). Prenatal diagnosis of tetrasomy 12p by *in situ* hybridization: varying levels of mosaicism in different fetal tissues, *Prenat. Diagn.*, **12,** 979–983.

Bresson, J.L., Arbez-Gindre, F., Peltie, J., Gouget, A. (1991). Pallister Killian–mosaic tetrasomy 12p syndrome. Another prenatally diagnosed case, *Prenat. Diagn.*, **11,** 271–275.

Chambers, H.M., Knowles, S., Staples, A., Tamblyn, M., Haan, E.A. (1993). Anthropometric measurements in the second trimester fetus, *Early Hum. Dev.*, **33,** 45–49.

Looijenga, L.H.J., Smit, V.T.H.B.M., Wessels, J.W., Mollevanger, P., Oosterhuis, J.W., Cornelisse, C.J., Devilee, P. (1990). Localization and polymorphism of a chromosome 12-specific α satellite DNA sequence, *Cytogenet. Cell Genet.*, **53,** 216–218.

McLean, S., Stanley, W., Stern, H., Fonda-Allen, J., Devine, G., Ellingham, T., Rosenbaum, K. (1992). Prenatal diagnosis of Pallister–Killian syndrome: resolution of cytogenetic ambiguity by use of fluorescent *in situ* hybridization, *Prenat. Diagn.*, **12,** 985–991.

Nagafuchi, S., Yanagisawa, H., Sato, K., *et al.* (1994). Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p, *Nature Genet.*, **6,** 14–18.

Narahara, K., Wakita, Y., Kikkawa, K., Hiramoto, K., Namba, H., Murakami, M., Kasai, R., Kimoto, H. (1988). Pallister–Killian syndrome: cytogenetic and biochemical studies, *Jpn. J. Hum. Genet.*, **33,** 339–347.

Pallister, P.D., Meisner, L.F., Elejalde, B.R., Francke, U., Herrmann, J., Spranger, J., Tiddy, W., Inhorn, S.L., Opitz, J.M. (1977). The Pallister mosaic syndrome, *Birth Defects: Orig. Artic. Ser.*, **XIII** (3B), 103–110.

Reynolds, J.F., Daniel, A., Kelly, T.E., Gollin, S.M., Stephan, M.J., Carey, J., Adkins, W.N., Webb, M.J., Char, F., Jimenez, J.F., Opitz, J.M. (1987). Isochromosome 12p mosaicism (Pallister mosaic aneuploidy or Pallister–Killian syndrome): report of 11 cases, *Am. J. Med. Genet.*, **27,** 257–274.

Rivera, H., Garcia-Esquivel, L., Jimenez-Sainz, M., Vaca, G., Ibarra, B., Cantu, J.M. (1987). Centric fission, centromere–telomere fusion and isochromosome formation: a possible origin of a *de novo* 12p trisomy, *Clin. Genet.*, **31,** 393–398.

Sachs, E.S., Van Hemel, J.O., den Hollander, J.C., Jahoda, M.G.J. (1987). Marker chromosomes in a series of 10 000 prenatal diagnoses. Cytogenetic and follow-up studies, *Prenat. Diagn.*, **7,** 81–89.

Schinzel, A. (1991). Tetrasomy 12p (Pallister–Killian syndrome), *J. Med. Genet.*, **28,** 122–125.

Sharland, M., Hill, L., Patel, R., Patton, M. (1991). Pallister–Killian syndrome diagnosed by chorionic villus sampling, *Prenat. Diagn.*, **11,** 477–479.

Shivashankar, L., Whitney, E., Colmorgen, G., Young, T., Munshi, G., Wilmoth, D., Byrne, K., Borgaonkar, D.S., Picciano, S.R., Martin-Deleon, P.A. (1988). Prenatal diagnosis of tetrasomy 47,XY,+i(12p) confirmed by *in situ* hybridization, *Prenat. Diagn.*, **8,** 85–91.

Speleman, F., Leroy, J.G., Van Roy, N., De Paepe, A., Suijkerbuijk, R., Brunner, H., Looijenga, L., Verschraegen-Spae M., Orye, E. (1991). Pallister–Killian syndrome: characterization of the isochromosome 12p by fluorescent *in situ* hybridization, *Am. J. Med. Genet.*, **41,** 381–387.

Steinbach, P., Rehder, H. (1987). Tetrasomy for the short arm of chromosome 12 with accessory isochromosome [+i(12p)] and a marked LDH-B gene dosage effect, *Clin. Genet.*, **32,** 1–4.

Stengel-Rutkowski, S., Albert, A., Murken, J.D., Zahn-Messow, K., Rodewald, A., Zankl, M., Saule, H., Sterne, J. (1981). New chromosomal dysmorphic syndromes, *Eur. J. Pediatr.*, **136,** 249–262.

Van Opstal, D., Eussen, H.J., Van Hemel, J.O., Sachs, E.S. (1993). Application of fluorescent *in situ* hybridization for '*de novo*' anomalies in prenatal diagnosis, *Prenat. Diagn.*, **13,** 825–832.

Warburton, D., Anyane-Yeboa, K., Francke, U. (1987). Mosaic tetrasomy 12p: four new cases, and confirmation of the chromosomal origin of the supernumerary chromosome in one of the original Pallister-mosaic syndrome cases, *Am. J. Med. Genet.*, **27,** 275–283.

Wilson, R.D., Harrison, K., Clarke, L.A., Yong, S.L. (1994). Tetrasomy 12p (Pallister–Killian syndrome): ultrasound indicators and confirmation by interphase FISH, *Prenat. Diagn.*, **14,** 787–792.

Zhang, J., Marynen, P., Devriendt, K., Fryns, J.P., Van den Berghe, H., Cassiman, J.J. (1989). Molecular analysis of the isochromosome 12p in the Pallister–Killian syndrome. Construction of a mouse–human hybrid cell line containing an i(12p) as the sole human chromosome, *Hum. Genet.*, **83,** 359–363.