PRENATAL DIAGNOSIS
Prenat. Diagn. **18:** 393–398 (1998)

SHORT COMMUNICATION

PRENATAL DIAGNOSIS OF *DE NOVO* ISOCHROMOSOME 13q ASSOCIATED WITH MICROCEPHALY, ALOBAR HOLOPROSENCEPHALY AND CEBOCEPHALY IN A FETUS

CHIH-PING CHEN[1,2]*, SCHU-RERN CHERN[2], CHEN-CHI LEE[3], LI-FENG CHEN[3], CHUN-YU CHUANG[2] AND MING-HONG CHEN[4]

[1]*Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan, Republic of China*
[2]*Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan, Republic of China*
[3]*Cytogenetic Laboratory, Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan, Republic of China*
[4]*Department of Pathology, Mackay Memorial Hospital, Taipei, Taiwan, Repubic of China*

Received 3 April 1997
Revised 4 August 1997
Accepted 10 August 1997

SUMMARY

We report on the prenatal diagnosis, genetic studies, and pathology of a case with *de novo* isochromosome 13q. A 31-year-old primigravida was referred for genetic counselling at 26 weeks' gestation due to the sonographic findings of intrauterine growth retardation and microcephaly. Level II ultrasonograms further demonstrated alobar holoprosencephaly, hypotelorism, polydactyly, a ventricular septal defect, and a single nostril. A diagnosis of cebocephaly was made. Genetic amniocentesis and cord blood sampling revealed translocation trisomy 13 with a *de novo* t(13q13q) rearrangement. Chromosomal analysis using G- and C-banding techniques and fluorescence *in situ* hybridization (FISH) showed an apparent monocentric isochromosome. Molecular analyses using polymorphic molecular markers showed that the rearrangement was consistent with an isochromosome of maternal chromosome 13q [46,XX,i(13)(q10)]. Necropsy confirmed cebocephaly and the prenatally detected anomalies. © 1998 John Wiley & Sons, Ltd.

KEY WORDS: isochromosome 13q; holoprosencephaly; cebocephaly; polymorphic molecular analysis; prenatal diagnosis; FISH

INTRODUCTION

Holoprosencephaly (HPE), with a spectrum encompassing alobar, semilobar, and lobar HPE, is a developmental abnormality of the brain resulting from failure of cleavage of the prosencephalon and is frequently accompanied by midline facial abnormalities such as cyclopia, ethmocephaly, cebocephaly, and premaxillary agenesis. In alobar HPE, the cerebral hemispheres are fused and enclose a single prosencephalic ventricle. In cebocephaly, literally 'monkey head', there are ocular hypotelorism and a blind-ended single nostril nose (Kjær *et al.*, 1991). We present a case of alobar holoprosencephaly and cebocephaly with a *de novo*

*Correspondence to: Chih-Ping Chen, MD, Department of Obstetrics and Gynecology, and Department of Medical Research, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Road, Taipei, Taiwan, Republic of China.

Contract grant sponsor: National Science Council, Taiwan, Republic of China; Contract grant number: NSC-86-2314-B-195-006.

CCC 0197–3851/98/040393–06 $17.50

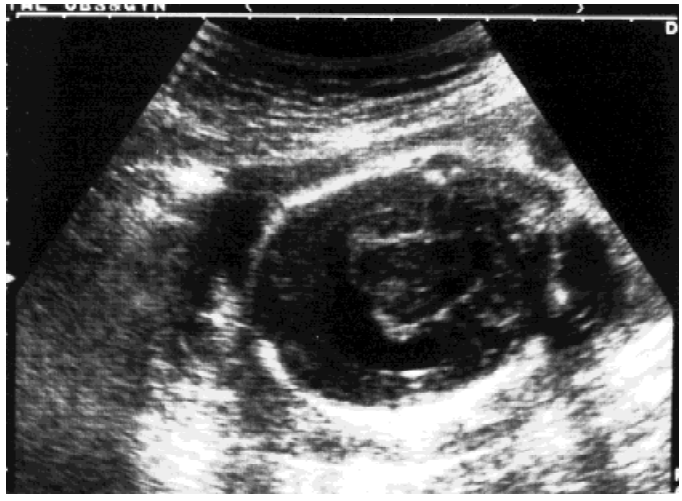

Fig. 1—Prenatal ultrasonography of the fetal brain at 26 weeks' gestation revealing centrally fused thalami surround by a monoventricle

homologous t(13q13q) rearrangement. The rearrangement was found to be an isochromosome with genetically identical long arms derived from a single maternal chromosome 13.

CASE REPORT

A 31-year-old primigravid mother was referred for genetic counselling at 26 weeks' gestation due to the sonographic findings of intrauterine growth retardation and microcephaly. She and her spouse were Chinese, non-consanguineous, and healthy. There was no family history of diabetes mellitus or congenital malformations. Maternal urine throughout the pregnancy did not contain glucose. The mother denied any exposure to alcohol, teratogenic agents, irradiation or infectious diseases during this pregnancy. At 16 weeks' gestation, the woman had a Down syndrome risk of 1:669, calculated from a maternal serum alpha-fetoprotein level of 0·65 multiples of the median (MOM) and a free β human chorionic gonadotrophin level of 1·38 MOM. Prior to 26 weeks' gestation, her pregnancy had been uneventful and routine sonographic examinations at a private clinic failed to detect any fetal abnormality. At 26 weeks' gestation, level II ultrasonograms at our hospital further manifested alobar HPE, centrally fused thalami surrounded by a monoventricle (Fig. 1), hypotelorism, a single nostril, microcephaly with a biparietal diameter equal to 21 weeks' gestation, a ventricular septal defect, and polydactyly. The diagnosis of cebocephaly was made. Genetic amniocentesis and cord blood analysis revealed translocation trisomy 13 with a *de novo* t(13q13q) rearrangement (Fig. 2). Chromosomal analysis using G- and C-banding techniques and fluorescence *in situ* hybridization (FISH) showed an apparent monocentric isochromosome (Fig. 3). Chromosome studies on the parents showed a 46,XY karyotype in the father and a 46,XX karyotype in the mother. Molecular analysis using polymorphic molecular markers confirmed that the rearrangement was consistent with an isochromosome of the maternal chromosome 13q [46,XX,i(13)(q10)]. She elected to terminate the pregnancy at 28 weeks' gestation. A female infant was delivered with a weight of 844 g and a length of 36 cm. On gross examination, the infant showed microcephaly, cebocephaly, ocular hypotelorism, a single nostril (Fig. 4), low-set ears, micrognathia, polydactyly, and rocker-bottom feet. At autopsy, the proband was found to have alobar HPE, arrhinencephaly, agenesis of the corpus callosum, a single ventricle of the brain, and a ventricular septal defect. Other internal organs were normal.

Fluorescence in situ *hybridization (FISH)*

FISH was carried out on metaphase chromosomes from the proband's lymphocytes using D21Z1/D13Z1 (Oncor), specific for the centro-

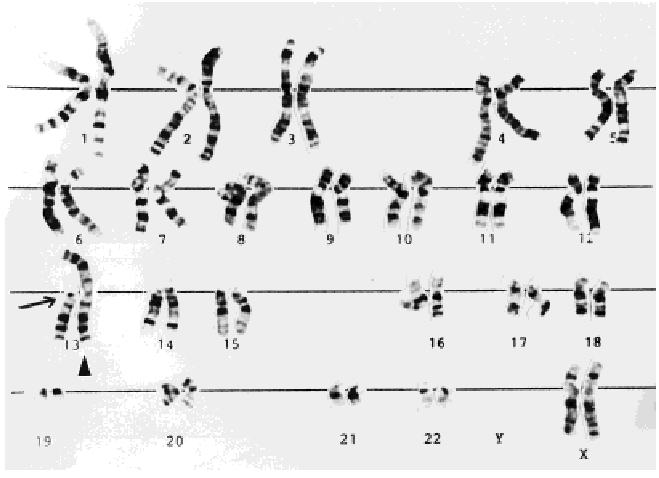

Fig. 2—The karyotype of the proband showing a normal chromosome 13 (arrow) and a t(13q13q) (arrow-head)

meres of chromosomes 13 and 21. The slides were dehydrated in a series of ethanol washes (70 per cent, 80 per cent, and 95 per cent) at room temperature for 2 min. The chromosomes were then air-dried. The slides were denatured in 70 per cent formamide/2 × SSC solution at 70°C for 2 min, immediately placed in a series of ethanol washes (70 per cent, 80 per cent, and 95 per cent) at −20°C for 2 min, and then air-dried. The alpha-satellite probes D21Z1/D13Z1 (Oncor) were obtained and labelled. The DNA probes were

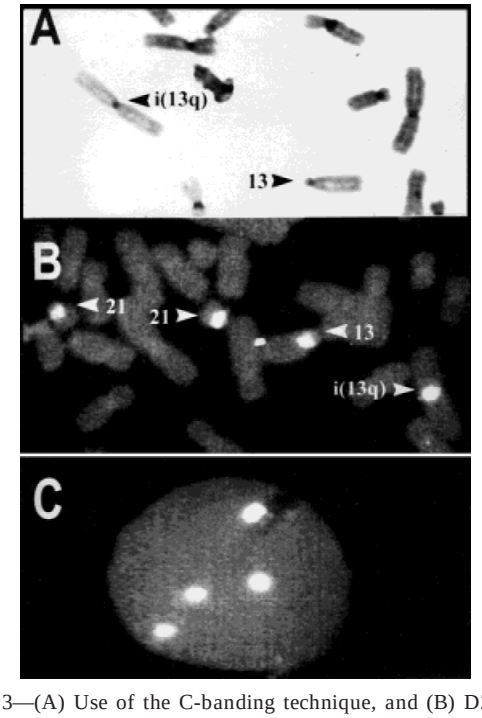

Fig. 3—(A) Use of the C-banding technique, and (B) D21Z1/D13Z1 (Oncor) alpha-satellite probes on metaphase cells showing an apparent monocentric isochromosome 13q. (C) An interphase nucleus showing four hybridization signals consistent with two chromosomes 21, a free-lying chromosome 13, and a monocentric i(13q)

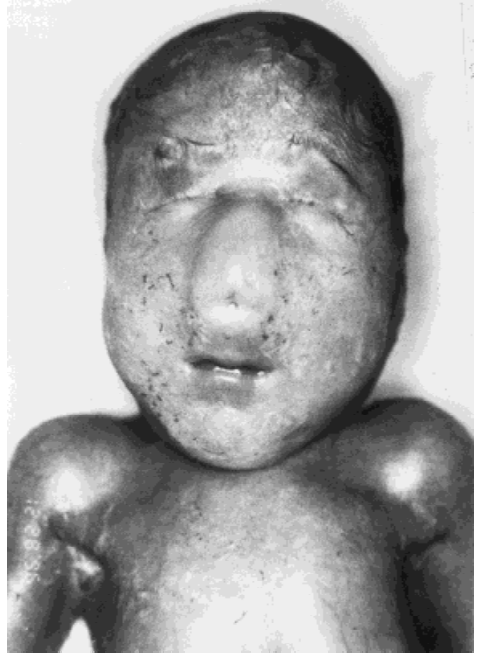

Fig. 4—Craniofacial dysmorphism of the proband

denatured at 70°C for 5 min, chilled on ice for 10 min, and hybridized on the prepared slides. The slides were incubated at 37°C overnight in a humidified chamber, washed in 0·5 × SSC solution at 72°C for 5 min without agitation, and then washed three times in 1 × PBD at 4°C for 2 min. The centromere DNA probes were detected using fluorescein isothiocyanate (FITC)-labelled anti-digoxigenin. The DNA was counterstained with propidium iodide in antifade solution. The signals were detected with a Zeiss Axioplan fluorescence microscope.

Genetic marker analysis

DNA was extracted from the cord blood of the proband and from blood samples of the parents using standard methodology. Nine polymorphic dinucleotide repeat markers (D13S115, D13S221, D13S263, D13S328, D13S262, D13S156, D13S154, D13S159, D13S280) for chromosome 13 were used to determine the parental origins of the rearrangement. The marker loci were based on microsatellite maps of chromosome 13 (Gyapay *et al*., 1994; Dib *et al*., 1996). To perform the polymerase chain reaction (PCR), 20 ng of genomic DNA was amplified in a 20 μl reaction mixture. Amplification was carried out on a 'DNA Thermal Cycler' (Perkin Elmer, U.S.A.) with 35 cycles of 95°C for 30 s and 60°C for 40 s. A 12 μl aliquot of the PCR products was analysed on 8 per cent sequencing gels. After silver staining and drying of the gels, the DNA bands were analysed by densitometry (UVP, U.S.A.) to estimate their intensities. The parental origins were determined by comparing the allele dosages. The informative markers D13S115, D13S263, D13S328, D13S262, D13S154, D13S159, and D13S280 showed homozygosity of maternal origin alleles. The markers D13S221 and D13S156 were uninformative. For the marker D13S115, the proband had apparently inherited two copies of the maternal allele 'a' and one copy of the paternal allele 'b' on the basis of band intensity. For the marker D13S280, the proband had apparently inherited two copies of the maternal allele 'c' and one copy of the paternal allele 'b'. Likewise, the proband had inherited double doses of the same allele from the mother and one allele from the father for the markers D13S263, D13S328, D13S262, D13S154 and D13S159 (Fig. 5). The results (Table I) were consistent with a maternal isochromosome.

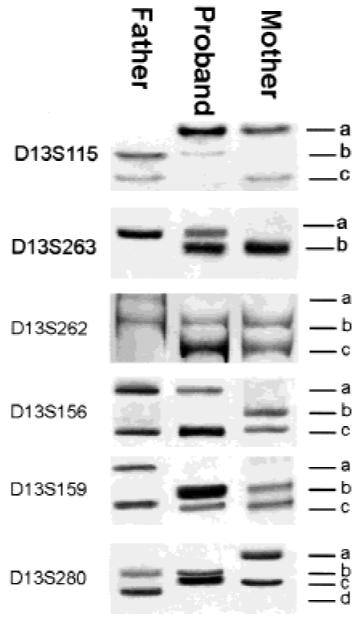

Fig. 5—Representative molecular results for isochromosome 13q of maternal origin. For the marker D13S115, the proband had apparently inherited two copies of the maternal allele 'a' and one copy of the paternal allele 'b' on the basis of band intensity. For the marker D13S280, the proband had apparently inherited two copies of the maternal allele 'c' and one copy of the paternal allele 'b'. Likewise, the proband had inherited double doses of the same allele from the mother and one allele from the father for the markers D13S263, D13S262, and D13S159. The marker D13S156 was uninformative

Table I—Microsatellite results for isochromosome 13q of maternal origin

Locus	Location	Father	Proband	Mother
D13S115	13q11–q12.1	b, c	a, a, b	a, c
D13S221	13q12.1	a, b	a, b, b	b, c
D13S263	13q14.1–q14.2	a, a	a, b, b	b, b
D13S328	13q14.2–q14.3	a, c	b, b, c	b, b
D13S262	13q14.3	a, b	b, c, c	b, c
D13S156	13q21.2–q22	a, c	a, c, c	b, c
D13S154	13q31–q32	b, b	a, a, b	a, c
D13S159	13q32	a, c	b, b, c	b, c
D13S280	13q32	b, d	b, c, c	a, c

DISCUSSION

To our knowledge, this is the first report of prenatally diagnosed isochromosome 13q associated with alobar holoprosencephaly and

cebocephaly. Cytogenetic abnormalities have been reported in half of HPE cases among live births (Ming *et al*., 1976; Muenke, 1994). HPE occurs in about 70 per cent of patients with trisomy 13 (Warkany *et al*., 1966; Taylor, 1968). Approximately 75 per cent of the cytogenetically abnormal cases of HPE are associated with trisomy 13 (Croen *et al*., 1996; Whiteford and Tolmie, 1996). Translocation trisomy 13 is associated with the typical Patau syndrome phenotype and occurs with an approximate frequency of 1/25 000 live births (Hook, 1980). About 25 per cent of spontaneous abortuses and the same proportion of liveborn infants with Patau syndrome have translocation trisomy 13. The t(13q14q) translocations make up most of the translocation Patau syndrome, while t(13q15q) and t(13q13q) comprise no more than 40 per cent of all cases with translocation trisomy 13 (Tolmie, 1997). A carrier parent with t(13q13q) can produce an abnormal conceptus that is either monosomic or trisomic for trisomy 13, or, very rarely, a normal offspring with uniparental isodisomy 13 (Slater *et al*., 1994; Stallard *et al*., 1995). Our case is a *de novo* translocation trisomy 13 since both parents have normal karyotypes. About 90 per cent of t(13q13q) cases have arisen *de novo* and the estimated mutation rate for *de novo* t(13q13q) is 0·5 per 10^5 gametes at conception (Hook, 1981). Translocation trisomy is associated with either isochromosomes or Robertsonian translocations. Most homologous rearrangements of t(21q21q) chromosomes have been shown to be isochromosomes and the parental origins of i(21q) have been equally divided between maternal and paternal origins (Shaffer *et al*., 1991, 1993). However, both parental origins of isochromosomes and maternal Robertsonian translocations have been reported in cases of t(13q13q) (Hassold *et al*., 1987; Shafer *et al*., 1994; Slater *et al*., 1994; Robinson *et al*., 1996). The *de novo* t(13q13q) in this case is more likely to be a maternal isochromosome 13q on the basis of proximal homozygosity and complete homozygosity without detected recombination at various loci of the chromosome 13q.

Translocation trisomy 13 such as *de novo* t(13q13q) associated with HPE is rare but not unexpected. The dysmorphic features of t(13q13q) translocation trisomy 13 have been consistent with classical presentation for trisomy 13 (Shaffer *et al*., 1994; Robinson *et al*., 1996). Croen *et al*. (1996) reported two cases with translocation trisomy 13 out of 38 cases of trisomy 13 in a total of 121 cases of HPE (50 cases were cytogenetically abnormal and 71 cases were cytogenetically normal) identified among a cohort of 1 035 386 live births and fetal deaths. Siebert *et al*. (1990) reported a case of t(13q13q) translocation trisomy 13 with a median cleft lip, palate and presumed HPE. Chervenak *et al*. (1985) reported a case of cebocephaly, alobar HPE, polydactyly, a micropenis, and a ventricular septal defect in a fetus with t(13q14q) translocation trisomy 13. Conen *et al*. (1966) reported a case of cebocephaly, HPE, polydactyly, and genitourinary defects in a case with t(DqDq), possibly translocation trisomy 13. Ming *et al*. (1976) reviewed 21 reported cases of cebocephaly and found 11 cases with various types of chromosome aberrations. Our case further demonstrates an association between *de novo* isochromosome 13q and cebocephaly.

ACKNOWLEDGEMENTS

This work was supported by a research grant (NSC-86-2314-B-195-006) to Chih-Ping Chen for fetal cerebral malformations from the National Science Council, Taiwan, Republic of China.

REFERENCES

Chervenak, F.A., Isaacson, G., Hobbins, J.C., Chitkara, U., Tortora, M., Berkowitz, R.L. (1985). Diagnosis and management of fetal holoprosencephaly, *Obstet. Gynecol.*, **66,** 322–326.

Conen, P.E., Erkman, B., Metaxotou, C. (1966). The 'D' syndrome. Report of four trisomic and one D/D translocation cases, *Am. J. Dis. Child.*, **111,** 236–247.

Croen, L.A., Shaw, G.M., Lammer, E.J. (1996). Holoprosencephaly: epidemiologic and clinical characteristics of a California population, *Am. J. Med. Genet.*, **64,** 465–472.

Dib, C., Fauré, S., Fizames, C., Samson, D., Drouot, N., Vignal, A., Millasseau, P., Marc, S., Hazan, J., Seboun, E., Lathrop, M., Gyapay, G., Morissette, J., Weissenbach, J. (1996). A comprehensive genetic map of the human genome based on 5264 microsatellites, *Nature*, **380,** 152–154.

Gyapay, G., Morissette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M., Weissenbach, J. (1994). The 1993–94 Généthon human genetic linkage map, *Nature Genet.*, **7,** 246–339.

Hassold, T., Jacobs, P.A., Leppert, M., Sheldon, M. (1987). Cytogenetic and molecular studies of trisomy 13, *J. Med. Genet.*, **24,** 725–732.

Hook, E.B. (1980). Rates of 47, +13 and 46 translocation D/13 Patau syndrome in live births and comparison with rates in fetal deaths and at amniocentesis, *Am. J. Hum. Genet.*, **32,** 849–858.

Hook, E.B. (1981). Unbalanced Robertsonian translocations associated with Down's syndrome or Patau's syndrome: chromosome subtype, proportion inherited, mutation rates, and sex ratio, *Hum. Genet.*, **59,** 235–239.

Kjær, I., Keeling, J.W., Græm, N. (1991). The midline craniofacial skeleton in holoprosencephalic fetuses, *J. Med. Genet.*, **28,** 846–855.

Ming, P.M., Goodner, D.M., Park, T.S. (1976). Cytogenetic variants in holoprosencephaly. Report of a case and review of the literature, *Am. J. Dis. Child.*, **130,** 864–867.

Muenke, M. (1994). Holoprosencephaly as a genetic model to study normal craniofacial development, *Semin. Dev. Biol.*, **5,** 293–301.

Robinson, W.P., Bernasconi, F., Dutly, F., Lefort, G., Romain, D.R., Binkert, F., Schinzel, A.A. (1996). Molecular studies of translocations and trisomy involving chromosome 13, *Am. J. Med. Genet.*, **61,** 158–163.

Shaffer, L.G., Jackson-Cook, C.K., Meyer, J.M., Brown, J.A., Spence, J.E. (1991). A molecular genetic approach to the identification of isochromosomes of chromosome 21, *Hum. Genet.*, **86,** 375–382.

Shaffer, L.G., McCaskill, C., Haller, V., Brown, J.A., Jackson-Cook, C.K. (1993). Further characterization of 19 cases of rea(21q21q) and delineation as isochromosomes or Robertsonian translocations in Down syndrome, *Am. J. Med. Genet.*, **47,** 1218–1222.

Shaffer, L.G., McCaskill, C., Han, J.Y., Choo, K.H., Cutillo, D.M., Donnenfeld, A.E., Weiss, L., van Dyke, D.L. (1994). Molecular characterization of *de novo* secondary trisomy 13, *Am. J. Hum. Genet.*, **55,** 968–974.

Siebert, J.R., Cohen, M.M., Jr, Sulik, K.K., Shaw, C.-M., Lemire, R.J. (1990). *Holoprosencephaly: An Overview and Atlas of Cases*, New York: Wiley–Liss.

Slater, H., Shaw, J.H., Dawson, G., Bankier, A., Forrest, S.M. (1994). Maternal uniparental disomy of chromosome 13 in a phenotypically normal child, *J. Med. Genet.*, **31,** 644–646.

Stallard, R., Krueger, S., James, R.S., Schwartz, S. (1995). Uniparental isodisomy 13 in a normal female due to transmission of a maternal t(13q13q), *Am. J. Med. Genet.*, **57,** 14–18.

Taylor, A.I. (1968). Autosomal trisomy syndromes: a detailed study of 27 cases of Edwards' syndrome and 27 cases of Patau's syndrome, *J. Med. Genet.*, **5,** 227–252.

Tolmie, J.L. (1997). Down syndrome and other autosomal trisomies. In: Rimoin, D.L., Connor, J.M., Pyeritz, R.E. (Eds). *Principles and Practice of Medical Genetics*, 3rd edn, New York: Churchill Livingstone, 925–971.

Warkany, J., Passarge, E., Smith, L.B. (1966). Congenital malformations in autosomal trisomy syndromes, *Am. J. Dis. Child.*, **112,** 502–517.

Whiteford, M.L., Tolmie, J.L. (1996). Holoprosencephaly in the west of Scotland, 1975–1994, *J. Med. Genet.*, **33,** 578–584.

