References

- ¹ Manzke H. Symmetrische hyperphalangie des zweiten fingers durch ein akzessorisches metacarpale. *Fortschr Röntgenstr* 1966; **105**:425–7.
- ² Catel W. *Differentialdiagnose von krankheitssymptomen bei kindern und jugendlichen*. Vol 1, 3rd ed. Stuttgart: Thieme, 1961:218–20.
- ³ Sundaram V, Taysi K, Hartmann AF, Shackleford GD, Keating JP. Hyperphalangy and clinodactyly of the index finger with Pierre-Robin anomaly: Catel-Manzke syndrome. A case report and review of the literature. *Clin Genet* 1982; **21**:407–10.
- ⁴ Silengo MC, Franceschini P, Cerutti A, Fabris C. Pierre Robin syndrome with hyperphalangism-clinodactylism of the index finger: a possible new palato-digital syndrome. *Pediatr Radiol* 1977; **6**:178–80.
- ⁵ Gewitz M, Dinwiddie R, Yuille T, Hill E, Carter CO. Cleft palate and accessory metacarpal of index finger syndrome: possible familial occurrence. *J Med Genet* 1978; **15**:162–3.
- ⁶ Stevenson RE, Taylor HA, Burton OM, Hearn HB. A digito-palatal syndrome with associated anomalies of the heart, face, and skeleton. *J Med Genet* 1980; **17**:238–41.
- ⁷ Holthusen W. The Pierre Robin syndrome: unusual associated developmental defects. *Ann Radiol (Paris)* 1972; **15**:253–62.
- ⁸ Farnsworth PB, Pacik PT. Glossoptotic hypoxia and micrognathia—the Pierre Robin sequence reviewed. *Clin Pediatr* 1971; **10**:600–6.
- ⁹ Lipson A, Beuhler B, Bartley J, et al. Maternal hyperphenylalaninaemia fetal effects. *J Pediatr* 1984; **104**:216–20.
- ¹⁰ Stevenson RE, Huntley CC. Congenital malformations in offspring of phenylketonuric mothers. *Pediatrics* 1967; **40**:33–45.

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Outcome after prenatal detection of a sporadic, unstable translocation t(5;21)

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SUMMARY Amniotic fluid cultures from a 37 year old woman showed a sporadic 46,XX,t(5;21)(5pter→5p13 or p14::5pter→5p13 or p14::21p12→21qter) complement. In the majority of metaphases the 5p fragment was attached to the stalks of chromosome 21; however, in 9% of metaphases, the fragment was loosely attached by a 'thread' and in 6% it was completely detached. Silver staining and in situ hybridisation with a homologous ribosomal gene probe, which localises to stalk regions (nucleolar organisers, NOR) of human acrocentric chromosomes, failed to show a reciprocal exchange. Prognosis was uncertain because the possibility that the 5p fragment might have been lost in some cell lines could not be excluded. Nonetheless, the parents elected to continue the pregnancy. The translocation was confirmed in blood specimens obtained both at birth and at 1 year of age and

showed similar instability. However, the proband shows no anomalies and is developing normally at 1 year.

Case report

The mother was a 37 year old Caucasian female in good health. Her three previous pregnancies resulted in two normal offspring and one first trimester miscarriage. She underwent amniocentesis for prenatal diagnosis at 18 weeks' gestation because of advanced maternal age. Family history was unremarkable except for one paternal uncle of the proband said to be 'slow', and one distant cousin said to have Down syndrome. Records were not available on these two people. Pregnancy had been unremarkable, there were no documented exposures to potential teratogens, and the only drugs taken were iron and vitamins. After the chromosomal aberration (to be described later) was detected in amniotic fluid cultures, detailed ultrasound examinations were performed at 21 and 29 weeks' gestation. There was no evidence of growth retardation or anomalies. Despite the uncertain prognosis,

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in particular because some fetal cells may have lost the 5p fragment, the parents elected to continue the pregnancy. Labour and delivery were uneventful. Apgar scores were 9 at one and five minutes. The female infant weighed 3.63 kg (90th centile), measured 53.5 cm in length (95th centile), and had a head circumference of 35 cm (75th centile). Physical examination revealed no anomalies.

During the first year of life growth and psychomotor development have been completely normal. The proband's only illness was a mild upper respiratory infection at 9 months of age. At her most recent physical examination at 1 year of age she weighed 10 kg (70th centile), measured 80 cm in length (95th centile), and had a head circumference of 47 cm (80th centile).

CYTOGENETIC ANALYSIS

Amniotic fluid cultures were initiated in routine fashion, which in our laboratory includes use of several types of culture media (Chang's, Ham's F12 in this case). Chromosomal analysis of GPG banded preparations two weeks later (pancreatin pretreatment followed by staining in Wright's Giemsa)

FIG 2 (a) The arrows indicate the appearance of the der(21) after silver staining and the der(5) which was Ag negative in all cells examined. (b) and (c) Association of der(21) with D and G group chromosomes as revealed by silver staining.

FIG 1 Translocation between 5p13 or p14 and 21p12 exhibiting morphological instability. (a) The portion from 5p appears firmly attached to 21p. (b) The portion from 5p is loosely attached to 21p. (c) The portion from 5p has become detached from 21p. (d) There is an association between der(21) and a chromosome 14.

revealed an unusual translocation between the stalk region of chromosome 21 and 5p13 or 14. All 94 cells from three cultures showed the two breakpoints. Usually (79 of 94 cells) the fragment from 5p was observed attached to the stalk region of chromosome 21 (fig 1a). In nine of the remaining 15 metaphases, it was attached loosely by a 'thread' to the stalks (fig 1b) and in six cells the fragment appeared completely detached, that is, appeared as an acentric fragment (fig 1c). The fragment was found in association with chromosome 14 in two metaphases (for example, fig 1d). Analysis of

50 cells from each of the parental peripheral blood cultures revealed no aberrations, nor any obvious 21 heteromorphisms (GPG banding) that would indicate the parental origin of the aberration.

After the infant was born, further studies were initiated. Cytogenetic analysis of 76 metaphases derived from cord blood cultures confirmed the presence of the aberration. In a single cell, however, only 45 chromosomes were present because the der(21) was absent. The morphological heterogeneity (instability) observed was similar to that seen in the cultures derived from amniotic fluid. In

FIG 3 Example of a metaphase after *in situ* hybridisation with a homologous 28S rDNA probe labelled with ^3H . Arrows indicate the der(5) and der(21). No label was ever observed in the der(5).

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addition, association of the der(21) with a chromosome 15 was observed in one of 76 cells. (RPMI 1640 and Ham's F12 media were used).

Silver staining (a modification of the method of Howell and Black¹) revealed an interstitial Ag positive region on der(21) (fig 2a). Silver bridges were also observed whenever the der(21) was associated with D or G group chromosomes (fig 2b). No positive silver staining of der(5) was observed in 15 cells exhibiting optimal silver staining, nor was the deleted chromosome 5 observed in association with an acrocentric chromosome (several hundred cells analysed).

In situ hybridisation to chromosomes was also performed using a pBR3 based recombinant plasmid containing 7.3 kbp of human genomic DNA, which is comprised of 0.2 kbp of the 3' end of the 18S rDNA, 2.5 kbp of internal transcribed spacer, 5.8 rDNA, and 4.6 kbp of the 28S rDNA gene. This plasmid was ³H labelled by nick translation.^{2,3} Slides were first treated with DNase free RNase (100 µg/ml) in 2 × SSC/1 mmol/l EDTA at 37°C for one hour. Following dehydration in a graded ethanol series (70%, 90%, 100%) they were air dried and stored in a dessicator. Chromosomal DNA was denatured at 70°C for two minutes in 70% deionised formamide (FA)/2 × SSC, and the slides were ethanol dehydrated and air dried. Hybridisations were performed using 50% FA/2 × SSC/10% (w/v) dextran sulphate, 250 µg/ml denatured and sonicated calf thymus DNA, and 200 ng/ml ³H plasmid (specific activity of approximately 2 × 10⁷ cpm/µg) at 42°C for 24 hours in a humidified chamber. Slides were washed 3 × 3 minutes in 50% FA/2 × SSC/42°C and 5 × 2 minutes in 2 × SSC/42°C, dehydrated, coated with nuclear track emulsion (Kodak NTB-2), and stored at 4°C in a dessicator. Samples were developed at two and three weeks, the latter providing optimal preparations. One hundred cells were scanned which showed definitive labelling of at least some of the acrocentric chromosomes and in which the der(5) could be identified by morphology (fig 3). In no cell was any label observed on der(5). Consequently, we were not able to demonstrate that the der(5) received a reciprocal exchange from chromosome 21. Presumably, parts of the stalk and attached satellites distal to the break in 21p12 were lost.

Discussion

The chromosomal rearrangement t(5;21)(5qter→5p13 or p14::5pter→5p13 or p14::21p12→21qter) detected initially in amniotic fluid cultures has several unusual features. One breakpoint was in the stalk of an acrocentric chromosome, creating an unstable

attachment of a fragment of 5p. This instability was presumably due to the interstitial NOR (stalk). Translocations of NORs appear to be rare events,⁴⁻⁷ indicating either that breaks are infrequent in stalks, or that if they occur, the result is unstable and consequently not detected. Indeed, stalks are not usually preserved in the relatively common Robertsonian translocations.⁸⁻¹² We are not aware of any previous reports of a morphologically unstable, acentric fragment with an attached stalk. Although the detachment of the 5p was occasionally observed, producing an unstable fragment, the piece was never lost. The in vitro observations may not reflect the in vivo situation, where the attachment could be either more or less stable.

A reciprocal exchange was not demonstrated; the broken end of 5p was presumably converted to a telomere, assuring a functionally stable chromosome configuration. Regeneration of telomeres is possible under the model that the composition of telomeres are repeats of 5'C₁₋₃A3^{1,13}.

Because of our concern that loss of the 5p fragment might occur in vivo, a normal phenotypic outcome could not be guaranteed to the parents. However, they decided to continue the pregnancy. Fortunately, the proband appears phenotypically normal; however, problems would be anticipated in her own future reproduction. Because we were not able to demonstrate a reciprocal exchange, and because only the 5p region could form a link between the chromosomes 5 and 21, pairing may be predominantly independent. If that were the case, 50% of the gametes would have the 5p region either deleted or in triplicate, both complements compatible with a viable, abnormal offspring. If the 5ps do indeed pair, the same type of unbalanced gametes would be expected from adjacent 1 segregation as from independent segregation. Although this is not a reciprocal translocation, the most likely segregation, based on criteria proposed by Jalbert and colleagues,¹⁴ seems to be adjacent 1 and 3:1. This prediction is based on the observations that (1) translocated and non-translocated segments are of unequal length; (2) an acrocentric is involved; (3) non-translocated segments are long relative to the translocated one; and (4) the translocated 'segments' are of unequal length (actually one is of 'zero' length because reciprocal exchange could not be demonstrated).

The frequency of unbalanced gametes might even be higher than predicted from expected segregation ratios, because the instability observed in vitro might occur during meiosis, causing potentially balanced gametes to be del(5p). Prenatal diagnosis would be recommended when the proband reaches reproductive age. Although her phenotype appears

normal, it seems that only the normal gamete with no derivative chromosome could safely be predicted to be normal phenotypically. The occurrence of a normal gamete would be at most 25%.

References

- ¹ Howell WM, Black DA. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a one-step method. *Experientia* 1980;**36**:1014-5.
- ² Erickson JM, Rushford CL, Darney DJ, Wilson GN, Schmickel RD. Structure and variation of human ribosomal DNA: molecular analysis of cloned fragments. *Gene* 1981;**16**:1-9.
- ³ Maniatis T, Jeffrey A, Kleid DG. Nucleotide sequence of the rightward operator of phage. *Proc Natl Acad Sci USA* 1975;**72**:1184-8.
- ⁴ Varley JM, Gosden J, Hultén M. Familial reciprocal translocation t(9;13) (p11;p12) investigated by silver staining and in situ hybridisation. *Hum Genet* 1981;**59**:422-8.
- ⁵ Watt JL, Couzin DA, Lloyd DJ, Stephen GS, McKay E. A familial insertion involving an active nucleolar organiser within chromosome 12. *J Med Genet* 1984;**21**:379-83.
- ⁶ Hansmann I, Wiedeking C, Grumin T, Gebauer J. Reciprocal and nonreciprocal chromosome translocations. *Hum Genet* 1977;**38**:1-5.
- ⁷ Dev VG, Byrne J, Bunch G. Partial translocation of NOR and its activity in a balanced carrier and in her cri-du-chat foetus. *Hum Genet* 1970;**51**:277-80.
- ⁸ Brasch JM, Smyth DR. Absence of silver bands in human Robertsonian translocation chromosomes. *Cytogenet Cell Genet* 1979;**24**:122-5.
- ⁹ Mattei MG, Mattei JF, Amymedel S, Giraud F. Dicentric Robertsonian translocation in man: 17 cases studied by R, C and N banding. *Hum Genet* 1979;**50**:33-8.
- ¹⁰ Gamberg N, Pajunen L, de la Chapelle A. NOR activity in two families with balanced D;D translocations and numerous consecutive miscarriages. *Hereditas* 1980;**92**:217-21.
- ¹¹ Mikkelsen M, Rasli A, Paulsen H. Nucleolar organizer regions in translocations involving acrocentric chromosomes. *Cytogenet Cell Genet* 1980;**26**:14-21.
- ¹² Gosden JR, Lawrie SS, Gosden CM. Satellite DNA sequences in the human acrocentric chromosomes: information from translocations and heteromorphisms. *Am J Hum Genet* 1981;**33**:243-51.
- ¹³ Szostak JW, Blackburn EH. Cloning yeast telomeres on linear plasmid vectors. *Cell* 1982;**29**:244-5.
- ¹⁴ Jalbert P, Sele B, Jalberg H. Reciprocal translocations: a way to predict the mode of imbalanced segregation by pachytene-diagram drawing. A study of 151 human translocations. *Hum Genet* 1980;**55**:209-22.

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