LETTERS TO THE EDITOR

Detection of a familial cryptic translocation by fluorescent in situ hybridisation

We read with great interest the report by Kohler *et al*¹ on a family with a half cryptic translocation involving chromosomes 9 and 17. In that report, three females in two generations had seemingly balanced translocations, and one male infant who died had exhibited features typical of Miller-Dieker syndrome. Additionally, two living male sibs, aged 20 and 17, had multiple congenital malformations as well as mental retardation. Chromosome analysis had shown the subtle translocation, which was further defined by FISH analysis using chromosome 17 specific probes.

Recently, we evaluated a family in which we eventually discovered a similar translocation involving chromosomes 17 and 22. The translocation was not detectable cytogenetically, even at a 850 band level. Because of a subtle clinical clue, however, it was discovered fortuitously by FISH analysis using chromosome 17 specific probes.

The proband was a 6 month old white male infant, born to healthy, non-consanguineous parents. The father was 35 years of age, and the mother was 33. The couple had two other healthy children, aged 4 and 3. The mother reported three early spontaneous miscarriages and one ectopic pregnancy. The only other significant family history was mild mental retardation in the mother's paternal uncle,

aged 49. The pregnancy was uneventful, until about 39 weeks of gestation, when the mother developed severe pre-eclampsia. Labour was induced one week later, with clinical signs suggesting that partial abruption of the placenta occurred immediately before delivery. The infant weighed 3355 g and the Apgar scores were 3 and 5 at one minute and five minutes, respectively. The infant was hypotonic, with a weak respiratory effort, and had to be intubated and transferred to a neonatal intensive care unit.

Our initial examination findings a few hours after birth included midfacial hypoplasia with a prominent nose, micrognathia, low set, incompletely rotated ears, and a small mouth with a high arched palate and wide alveolar ridges. Also, excess soft tissue was noted around the neck, which showed creasing bilaterally. Other findings included premature closure of the metopic suture, syndactyly between the third and fourth fingers on the left, bilateral simian creases, adducted thumbs, laterally displaced nipples, penile attachment high on the scrotum, rocker bottom feet, second toe overriding the third, and hypoplastic toenails. He had generalised muscular hypotonia and hyperactive deep tendon reflexes. Cardiac evaluation revealed tricuspid insufficiency, and an unusual aortic arch in which the transverse aortic section connected with the ascending and descending aortic sections at acute right angles. He had severe anaemia, for which idiopathic haemsiderosis was considered as a diagnostic possibility but could not be proven. Our clinical impression was that this patient probably had a triploidy/diploidy mosaicism; however, routine chromosome analysis showed a 46,XY karyotype.

An MRI study of the brain at 3 months of age showed agenesis of the corpus callosum, unusual Sylvian fissures, and probable polymicrogyria. As a result of these findings, the possibility of Miller-Dieker syndrome was

considered. High resolution chromosome analysis at the 850 band level was again normal. Then, FISH analysis was carried out using the probe D17S379 for Miller-Dieker syndrome at 17p13.3 (ONCOR). Clear signals were seen on both chromosomes 17p as well as at the control locus, RARA, and an additional signal for D17S379 was seen on the q terminus of a G group chromosome (figure). This chromosome was identified as a 22 by GTL banding, and a 22q deletion was confirmed by FISH using a D22S39 probe at 22q13.3 (ONCOR). FISH analysis was also carried out on both parents using the same probes, and a balanced translocation between 17p13.3 and 22q13.3 was found in the mother. This rearrangement was not detectable by high resolution chromosome analysis. The karyotype of the proband, therefore, is 46,XY,-22,+der(22),t(17;22)(p13.3;q13.3) mat. No cell line is available.

Unfortunately, the infant continued to have problems with anaemia and pneumonia, and died at 6 months of age. However, we are now able to provide genetic counselling to this family, and offer prenatal diagnosis using FISH analysis in the mother's future pregnancies, and test her two apparently normal children and other family members for carrier status.

We thank the parents for their cooperation and Drs A J Yazdi and W L Jackson for referring the family.

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1 Kohler A, Hain J, Muller U. Familial half cryptic translocation t(9;17). *J Med Genet* 1994;31: 712-14.

Chromosome fragments with alphoid sequences derived from a pseudoisodicentric Y chromosome

Isodicentric Y chromosomes constitute a relatively common form of normal length, non-fluorescent Y chromosomes. They probably originate from an isochromatid breakage in the Yq euchromatin followed by rejoining of the broken chromatids in the male germline. Their symmetrical appearance would result from centromere division in meiosis.¹ Habitually, but not always, one of the resulting centromeres is inactivated, thereby allowing normal segregation. They commonly occur in mosaicism with a 45,X cell line, and the associated phenotypic anomalies can be heterogeneous depending on the frequency of the 45,X cell line and the localisation of breakpoints. Thus, they can be diagnosed in patients with Turner's syndrome, ambiguous genitalia, apparently normal males, azoospermic males, or autistic children.¹⁻⁴

Accurate identification was not easy in the past, and G11 banding offered the best results. Nowadays, fluorescence in situ hybridisation (FISH), usually with specific centromeric Y alphoid sequences, allows these chromosome aberrations to be identified precisely.⁴⁻⁶ Demonstration of Y specific sequences may be relevant to therapeutics since gonadal dysgenesis patients with Y chromosome material have a 15-20% risk of developing gonadal neoplasia, gonadectomy usually being recommended.⁷

FISH analysis using ONCOR probe for Miller-Dieker syndrome (D17S379) showing additional signal on the derivative chromosome 22 (right centre arrow).

FISH of Y specific alphoid sequences on a pseudoisodicentric Y chromosome. (a) Two hybridisation signals appear symmetrical showing a dicentric nature. Examination under propidium iodide filter (b) of the chromosome shown in (c) allows a unique centromeric constriction (arrow) to be discriminated, the other centromere being inactivated. This fact is better appreciated after digital image analysis of the chromosome presented in (d). (propidium iodide filter) and (e) (FITC filter). (g) Besides the isodicentric, this mitosis shows a small chromosome fragment with Y specific alphoid sequences. (f) Its chromatin nature is evident in propidium iodide staining. (h) An insert showing two derived chromosome fragments.

In a previous report, a pseudoisodicentric Y chromosome was analysed in a 14 year old girl with signs of Turner's syndrome and virilisation.⁸ In this letter we present a new case of pseudoisodicentric Y chromosome, showing particular features. A 64 year old woman was referred because of somatic features of Turner's syndrome including primary amenorrhoea, small stature, and raised gonadotrophins accompanied by aortic aneurysm and clitorimegaly. Her karyotype was a mosaic 45,X/46,X+mar. FISH with a Y specific classical satellite DNA probe, DYZ1, failed to give a hybridisation signal, while a centromeric alphoid probe, DYZ3, confirmed 26% of nuclei to have Y sequences, and the marker chromosome was identified as an isodicentric Y, showing two areas of hybridisation in a symmetrical position (figure a,c,e). One centromere was inactivated in all markers examined, since only one primary constriction was evident (figure b,d,e). The alphoid signals were very close to each other so the breakpoint should be localised in the Yq arm, closer to the centromere than in our previously described case. The striking finding was that 6% of mitoses with isodicentrics had very small chromosome fragments with Y specific alphoid sequences (figure f,g,h). Given their low frequency, their presence is difficult to detect and identify with accuracy by current karyotypic analysis without FISH. Though other sequences could be integrated, most of their material seems to be composed of alphoid Y sequences.

The possible function of these fragments, as active centromeres, is difficult to assess. They probably originate in the inactive centromeric area of the pseudoisodicentric, since active centromeres from normal Y chromosomes obviously do not develop such structures. The fact that their number changes among different mitoses (one or two fragments) (figure g,h) also suggests that they

behave as inactive centromeres. The mechanism of generation of these structures is intriguing. Their morphology resembles double minute-like microchromosomes. Experimentally, the same kind of microchromosomes have been obtained after introduction of human alphoid DNA sequences into simian cells.⁹ In these studies, transfected alphoid sequences experienced amplification and gave rise to extrachromosomal elements. Our inactive centromeric area possibly exhibits some instability, showing disturbances in replication, or recombination events, that lead to amplification and fragility of alphoid sequences. This could explain the coexistence of the fragments with the isodicentric Y within the same mitoses.

It is now accepted that a functional human centromere is a complex structure where alphoid DNA sequence arrays and possibly other sequences are organised with specific centromeric proteins, like CENP-B, constituting specific higher order chromatin folding structures.¹⁰ In fact, partial or complete absence of centromere specific proteins,^{11,12} chromatin deletion, or changes in chromatin conformation have been reported in inactive centromeres.¹³ Nevertheless, since a large block of transfected and chromosome integrated alphoid DNA does not always form an active centromere, it is possible that additional genetic or epigenetic factors are required for activity.¹⁴ In some cases disruption of these factors might account for instability in certain inactivated centromeric regions.

From the clinical point of view, the presence of this very low frequency of mitoses with fragments does not appear to be of great practical consequence. Our patient was a relatively old woman when diagnosed, and gonadoblastoma has not developed, taking into account that most of these tumours arise between 15 and 30 years.⁵ The presumed Y linked gonadoblastoma locus⁷ may have been

deleted in the rearrangement. It would be of interest to see if this phenomenon were evident in similar cases or with other isodicentric chromosomes, or if it is merely an isolated observation. Interestingly, coexistence of a supernumerary microchromosome of unknown origin with a Yq isodicentric within the same mitosis was described in a case report by Haaf and Schmid.¹² They also observed this phenomenon in a very low proportion of mitoses. Insights into the organisation and mechanism of generation of such structures could produce interesting data about centromeric structure and physiology.

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Familial predisposition to both male and female germ cell tumours?

A minority of testicular teratomas are recognised to be familial.¹ Some occur as part of the spectrum of cancers in the Li-Fraumeni syndrome; however, the genetic basis of the majority of familial cases is unknown. This has prompted the formation of a Linkage Consortium to identify genes causing testicular teratomas. As one of the participating centres we have been ascertaining familial cases from a registry of testicular teratoma patients at the Royal Marsden Hospitals. Among these cases we have identified three families which suggest that a common genetic basis exists between some male and female germ cell tumours.

The first family was identified through an index case who presented with a seminoma at the age of 51, his brother had had a testicular teratoma at the age of 28, and their cousin an endodermal sinus tumour of the ovary diagnosed at 32 years. In the second family the index case presented with an undifferentiated malignant teratoma at 28 years of age and his sister was diagnosed with bilateral mature teratomatous cysts at the age of 39. In the third family the index case presented with a retroperitoneal teratoma at 26 years and his sister was diagnosed with an ovarian dysgerminoma at 45 years. None of these families had any features indicative of the Li-Fraumeni syndrome or any other cancer family syndrome, suggesting the identification of a previously unrecognised association. This is supported by two reports of single families with ovarian and testicular germ cell tumours^{2,3} and a family with multiple cases of dysgerminoma.⁴ The three families we report were identified from a

database of 2000 teratoma patients, suggesting that in 0.2% of pedigrees a female member will develop a germ cell tumour. This may be an underestimate since pedigree information on all 2000 index cases has not been verified and many mature teratomatous cysts are asymptomatic and go undiagnosed.

Whether an association between male and female germ cell tumours is the result of the inheritance of a single gene with effects on both ovary and testis or a consequence of the action of modifying genes will only be established when the gene or genes causing testicular teratomas are identified.

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BOOK REVIEWS

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DNA Cloning 2: A Practical Approach. Expression Systems. Editors D M Glover, B D Hames. (Pp 255; £25.00.) Oxford: IRL Press. 1995. ISBN 0-19-963478-5.

The many advances and widespread use of prokaryotic and eukaryotic protein expression systems is reflected in the increase in the size of this text, from four chapters in the first edition 10 years ago, to a complete volume in 1995 with an additional volume dedicated to mammalian systems. This volume provides a clearly written text covering the major aspects of protein expression, from the isolation of a cDNA through to the purification of the

expressed protein product and the generation of antibodies to that product. Systems covered include fusion and non-fusion protein expression in *E coli*, protein production, two hybrid interaction screening in yeast, and baculovirus expression of protein in yeast and insect cells. The generation of polyclonal and monoclonal antibodies to expressed proteins is also covered in some detail. The text provides sufficient introduction and background for a complete understanding of the methodology under discussion, with detailed protocols presented in clearly marked boxes within the text, often containing handy notes and tips from the authors' own laboratories. This volume would be entirely sufficient for someone outside the field to isolate a cDNA to a protein of interest, clone into an appropriate expression vector, achieve soluble protein expression, and raise antibodies as well as investigate possible other protein-protein interactions. It is more than a list of protocols, it provides a concise account of the "whys and wherefores" of the methods being used. As the individual chapters "stand alone", there is some duplication where the reader is given methods for SDS-PAGE or western blotting in more than one chapter, but this is a minor irritation. The only aspect of protein expression that is not covered, neither in this volume nor apparently in volume 4, is the production of protein in rabbit reticulocyte lysates, a rapid method for the generation of small quantities of correctly folded protein (often labelled) for the determination of protein-protein interactions or other functional assay. It is inevitable, however, as the editors hope, that this book will become "messy and dog-eared" from constant use in the laboratory, both as a source of reference and as a practical guide.

STEVE WINDER

Developmental Mechanisms of Heart Disease. Editors E B Clark, R R Markwald, A Takao. (Pp 679; \$159.00.) New York: Futura Publishing Company Inc. 1995.

The 4th International Symposium on Etiology and Morphogenesis of Congenital Heart Disease was held in Tokyo in November 1993. The editors' stated aim in this volume is to allow the participants of the symposium to share their knowledge with a broader audience of scientists and clinicians. The book includes the symposium papers as well as commissioned overview chapters and has been divided into six main sections and 72 papers or overview chapters.

The first section introduces molecular biology and the molecular analysis of cardiac developmental phenotypes including gene expression during embryonic development. The second section extends the application of molecular biology to medical genetics especially the cardiomyopathies. The third and largest section overall is divided into four overviews and 26 papers related to wider ranging aspects of cardiogenesis including regulation of chick and rat heart development as well as teratogenic cardiac abnormality. Aspects of the functional development of the heart are contained in the final section.

The penultimate section entitled "Human and Animal Models of Cardiac Defects" is of particular interest to clinicians covering conotruncal abnormalities and lateralisation defects as well as an interesting review of