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An analphoid supernumerary marker chromosome derived from chromosome 3 ascertained in a fetus with multiple malformations

EDITOR—We report a case in which a termination of pregnancy for fetal abnormality at 18 weeks' gestation showed a supernumerary marker chromosome. This extra chromosome did not hybridise to any alphoid probes and was found to have a chromosome 3 origin when investigated by M-FISH.

An anomaly ultrasound scan was performed because of raised alphafetoprotein and beta HCG levels at 17 weeks' gestation in a 32 year old, primigravida mother. The scan showed a large and cystic left kidney, banana sign, and absent cisterna magna, and signs of an open sacral spina bifida. The pregnancy was terminated and necropsy showed a male fetus consistent with 18 weeks' gestation with no dysmorphic facial features. A high arched palate with a small amount of postnuchal oedema was noted as well as a single transverse palmar crease on the right hand. Inspection of the back showed a 1.3 cm long lumbosacral myelomeningocele with protruding lower lumbar spinal cord. On internal examination the cerebral hemispheres were fully cleaved and appeared fluctuant suggesting the possibility of internal hydrocephalus. The posterior fossa of the brain was reduced in anteroposterior diameter as well as appearing deep and funnel shaped, and the extension of the cerebellar tonsils was below the level of the foramen magnum. These findings are consistent with Arnold-Chiari malformation. There was marked asymmetry of the kidneys; the right kidney showed normal fetal lobation and shape but the left kidney was very large and had thin, translucent, subcapsular cysts, especially at the lower pole. The cut surface showed a poor demarcation between the cortex and medulla and the presence of cysts in most of the renal parenchyma. These findings are consistent with cystic renal dysplasia. The placenta was unremarkable and the cord had three normal blood vessels.

The chromosomes of the abortus were examined from fetal skin fibroblasts derived using the method of Fisher *et al*.[1] The metaphases from the fetal fibroblasts and parental blood were GTL banded using a modification of the method of Seabright.[2] The abortus showed a male karyotype with a metacentric supernumerary marker chromosome approximately the size of a G group chromosome in 17 out of 30 (57%) metaphases examined in primary cultures. In subsequent passaging of the cultures, the proportion of the cells with the marker rapidly diminished. Both parents had apparently normal karyotypes. A fibroblast cell line (DD3329) and lymphoblastoid cell lines (DD3389 father, DD3390 mother) from both parents are available from ECACC, Porton Down, Salisbury, Wilts, SP4 0JG, UK.

Fluorescence in situ hybridisation (FISH) with nick translated biotin or digoxigenin (Boehringer-Mannheim UK) labelled centromere specific alpha satellite probes were used based on a technique by Pinkel *et al*.[3] The in situ hybridisation was detected using one layer of FITC conjugated anti-avidin for biotin labelled probes or TRITC conjugated anti-digoxigenin for digoxigenin labelled probes. Diamino-2-phenylindole (DAPI) at the rate of 0.05 mg/ml suspended in an antifade solution (Vectashield, Vector Labs, UK) was used to counterstain the chromosomes. A Carl Zeiss Axioskop epifluorescent microscope fitted with a Pinkel Fluorescent No 83 filter series (Chroma Technology) was used to examine the hybridisation, while a cooled charged couple device camera captured the images. Smartcapture software (Digital Scientific, Cambridge, UK) was used to analyse and visualise the digitised data. The normal homologues acted as internal controls for the FISH. The marker was screened with a library of alphoid centromere specific probes at 1 × SSC in 50% formamide stringency, but failed to hybridise to any of the probes, suggesting that what appeared to be the marker's primary constriction did not contain alphoid repeats. This was confirmed when an all centromere alphoid mixture used at low stringency (2 × SCC at room temperature) showed strong signal at all centromeres except for the marker (fig 1a).

Multiplex fluorescence in situ hybridisation (M-FISH)[4] was performed on the marker using the Spectra Vision Assay™ (Vysis). The protocol and probe set was as specified in the SpectraVision™ Assay protocol. The images were captured on a Provis microscope (Olympus) equipped with a motorised eight position turret with an epifluorescence filter set designed for the fluors used. Analysis was performed using M-FISH software supplied by Perceptive Scientific International Ltd (PSI). Using M-FISH, the marker was identified as being from chromosome 3.

FISH with the 3p and 3q subtelomere probes showed hybridisation to the 3q subtelomere probe on the ends of both arms (fig 1b), and wcp3 hybridised to the whole of the marker (fig 1c). Subsequently CGH[5 6] was applied using DNA extracted from fetal skin and testis. The CGH profiles were analysed using Vysis Quips CGH software following hybridisations to 10 metaphases from each tissue. The CGH profiles showed a significant gain of material in distal 3q26 in fetal skin and in DNA extracted from testis, a tissue not cultured in vitro. These profiles suggested that the tetrasomy may not include the most distal 3q bands (q28 and q29); however, CGH profiles at the extreme ends of chromosomes are known to be problematical because of variable repeat sequences. Conventional FISH with YAC 919f12 (3q29) confirmed that the marker contained two copies of this sequence (fig 1d). Owing to the instability of the marker in culture, we were unable to perform any investigations with constitutive centromere binding proteins. The conventional cytogenetics was re-evaluated and suggested that the marker was an inverted duplication from chromosome region 3q26.2→qter.

Molecular analysis was undertaken to check for the biparental inheritance of the two normal chromosome 3 homologues and to find the parental origin of the marker chromosome. DNA was extracted from fetal tissue and peripheral blood from the parents. Primer sets were used to detect polymorphic microsatellite repeat sequences along the length of chromosome 3 and it was found that the marker was maternal in origin and that the fetus had inherited one normal chromosome 3 from each of his parents and so excluded uniparental disomy 3.

As far as we are aware, the marker described here is the first instance of an inverted duplication causing tetrasomy for chromosome region 3q26.2→qter. Our patient had a prenatally detected lumbosacral myelomeningocele, Arnold-Chiari malformation with possible hydrocephalus, and cystic renal dysplasia, and as a result was terminated at 18 weeks' gestation. Arnold-Chiari malformation is seen in approximately 1 in 1000 livebirths[7] and is often associated

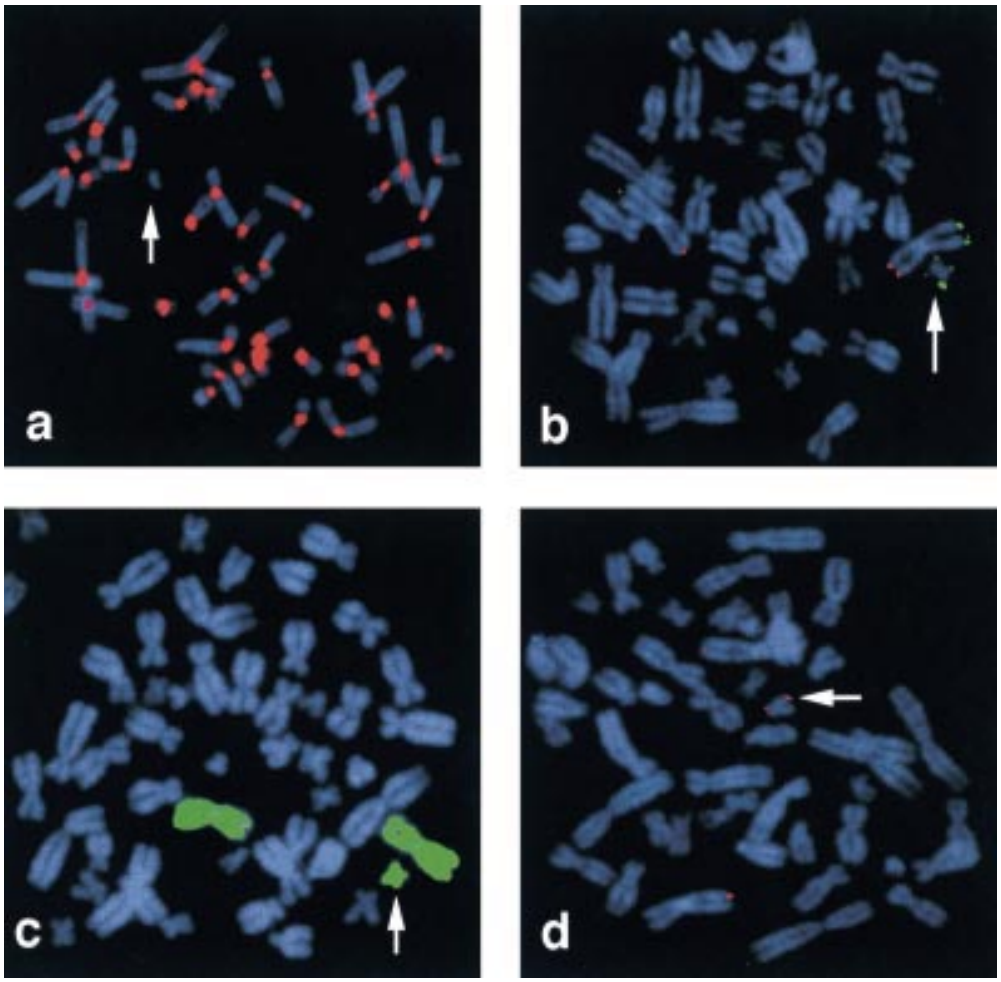

Figure 1 Molecular cytogenetic characterisation of the marker chromosome. Arrow indicates marker chromosome. (a) All centromere alphoid mix. (b) 3q subtelomere probe (196f4) green, 3p subtelomere probe (dJ11286B18) red. (c) Whole chromosome paint 3. (d) 3q29 probe (YAC 919f12).

with spina bifida with myelomeningocele and hydrocephalus. Schinzel[8] observed lumbosacral myelomeningocele and Arnold-Chiari malformation in single incidences of dup 3q23→25→q27 to qter. The marker breakpoint is thought to be at 3q26.2, so the marker contains two copies of 3q26.3, which Ireland *et al*[9] considered to be the location of the Cornelia de Lange syndrome gene and the duplication 3q syndrome critical region.[10] The only features seen which may be associated with de Lange or duplication 3q syndromes, and may also be coincidental, were high arched palate, a transverse crease on the right hand, and left cystic renal aplasia.[11] However, our case does prove the need to do a detailed karyotype where upper and mid neural tube defects are associated with other abnormalities.

Portnoï *et al*[12] reported a similar supernumerary marker chromosome in a healthy 22 year old male of normal intelligence. He was not dysmorphic, but was referred because of skin pigmentary anomalies showing hyperpigmented brown macular streaks following the lines of Blaschko, the onset of which occurred aged 10 to 12 years. The normal skin fibroblasts showed no evidence of the marker, but blood and hyperpigmented fibroblasts showed 30% and 6% cells respectively with the marker. Their marker was analphoid, acrocentric with a breakpoint 3q27.1. The lower level of mosaicism in vivo, proven tissue specificity, and the smaller size of the marker may account for the ameliorated phenotype in this patient compared with our fetus. Another neocentromere located at 3q26 was reported by Wandell *et al*[13] and was observed in a father and daughter, ascertained because of developmental delay in the child along with hypertelorism, epicanthus, and a large head. The father had borderline mental retardation. In this case the normal centromeric region was deleted from the chromosome 3 and had formed a small linear marker chromosome. The two distal portions of the deleted 3 had rejoined and a neocentromere was present at 3q26. Interestingly, the neocentromere formed microtubule associated kinetochores of the same size as other large chromosome kinetochores, but was found to be weakly positive with anticentromere (CREST) antibodies, whereas the normal centromere on the small marker chromosome showed a reduced kinetochore size but a strong CREST antibody signal.

Our marker increases the haploid autosomal length of the cell by about 1.5%, but is mosaic (57%) in primary cultures. Other analphoid markers which give rise to tetrasomies of the duplicated regions are also found to be unstable in long term or fibroblasts cultures and are often lost altogether.[14] It seems remarkable that these markers seem to be stable for many cell generations in vivo only to be lost so rapidly in culture. This instability in vitro makes it difficult to judge how much effect our marker had on the phenotype, although we know that it was present in tissues from two different embryonic lineages (fetal skin and testis).

The centromere is an essential structure of the chromosome and chromosomes lacking an active centromere will eventually be lost during subsequent cell divisions. The

centromeric DNA is composed of highly repetitive A+T rich sequences. The most investigated is alpha satellite DNA which in humans is a 171 bp sequence tandemly repeated many times such that between 2 and 4 Mb may be present in a typical centromere.[15] There seems to be no similarity in the primary DNA sequence between species and a lack of centromeric DNA conservation throughout evolution makes it difficult to equate its sequence to function.[16] Nonetheless, the repetitive nature of the DNA and its A+T content appears to be a consistent feature of many organisms and suggests that it is significant in centromere function.[17]

Supernumerary marker chromosomes (SMCs) have a prevalence of less than 1 in 1000 in the general population[18] and in recent years in situ hybridisation using alpha satellite probes allows the origin of most of the SMCs to be identified. However, a minority do not hybridise to any of the alphoid probes,[19] but nevertheless these analphoid markers are more or less stable in vivo and in vitro,[14 20] suggesting the presence of some centromeric properties, unlike a true acentric chromosome. Two main explanations have been suggested. Firstly, a complex rearrangement has deleted the normal centromere to such an extent that, although it can still function, the highly repetitive alpha satellite probes cannot hybridise to it. Secondly, when the normal centromere was lost, a latent centromere (or neocentromere) was activated in a region not normally associated with centromeric function.[16 21 22] This latter explanation is currently more favoured. Unfortunately, as marker chromosomes tend to be found by chance, only the endpoint is seen, never the intermediate steps nor the mechanism in action by which the neocentromere may be formed.[23]

Recent sequencing of the centromeric region of a chromosome 10 derived analphoid marker has shown that compared with the sequence of a normal centromere the marker centromere is lacking in repetitive sequences. The evidence from this neocentromere, and that from the deactivation of centromeres in dicentric chromosomes, is more proof that repetitive sequences per se do not dictate centromere function. In sequencing the chromosome 10 neocentromere, it was found that although the A-T content was no different from that of the rest of the genome, there was evidence of A-T rich islands but the significance of this remains unknown. Nor did the neocentromere sequence differ significantly from the homologous region in the normal chromosome 10 and there was no major feature present similar to known centromeric DNA. The complex rearrangement hypothesis seems unlikely because so much of the material that makes up a normal centromere is missing. So it does seem that a neocentromere forms from a latent centromere activating in a region not known to be centromeric.

Fewer than 35 neocentromeric markers have been reported to date, but they are probably more frequent than this figure suggests because of past difficulties in identification, and it is likely more will be recognised and characterised in the future.[14] Although at least 11 different chromosomes have been implicated in the formation of neocentromeres, it is interesting that our case is the third to be reported in which a neocentromere has been activated in the region near 3q26. Alphoid SMCs have duplicated material from around the centromere, whereas neocentromeric markers will allow us to investigate the effects of duplicated genetic material from other chromosomal regions.

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ANNETTE E COCKWELL*
BARBARA GIBBONS†
ISABELLA E MOORE‡
JOHN A CROLLA*

**Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, Wiltshire SP2 8BJ, UK*

†*NE Thames Regional Cytogenetics Laboratories, 2nd Floor Queen Square House, Institute of Neurology, Queen Square, London WC1N 3BG, UK*

‡*Histopathology Department, Level E, Southampton General Hospital, Tremona Road, Southampton SO9 4XY, UK*

Correspondence to: Dr Cockwell, wessex.genetics@dial.pipex.com

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Why patients do not attend for their appointments at a genetics clinic

EDITOR—When a patient does not attend a scheduled appointment, or cancels so late that a replacement cannot be found, there is a cost to the health care system in terms of personnel time, extended waiting lists, and the loss of potentially beneficial services to patients who miss their visit. These costs are particularly important for genetics clinics because a great deal of preparation is often required before a clinic visit. Preparation may include sending out a family history questionnaire from which a pedigree diagram is constructed, and a review of the medical reports and charts of the patient and other family members. In the case of rare genetic conditions, a preliminary review of publications/computer database search may be conducted and research laboratories may be sought which would be willing to receive patient samples. Furthermore, genetics departments typically set aside at least an hour for each new patient visit.

Failed appointment rates at community and university medical clinics have been reported to range between 10 and 30%.[1 2] Studies involving hospital clinics set in low socioeconomic status populations have shown no show rates in the upper end of this range, whereas family practice clinics have reported fail rates as low as 5%.[3]

There is some evidence to suggest that missed appointments may be more likely among certain demographic groups, such as young adults and adults with young children,[3] patients with lower socioeconomic and educational status, and those with larger families.[1] Moreover, geographical distance from the clinic or the inability to obtain transport or both have been found to impede appointment keeping.[1] Sex and race have not been associated with compliance.[1]

Problems with communicating to patients about the timing or nature of an appointment and in providing them with information about their diagnosis may lead to missed appointments,[1 4] and a strong recommendation by the referring physician has been shown to have a major benefit on compliance.[5–7]

There may be a relationship between clinic attendance rates and certain attitudinal factors. There is evidence that patients are more likely to miss their appointments if they perceive the appointment as less urgent[1 3] or less helpful.[4] Other potential psychological determinants of health care use are variables from the Health Belief Model (HBM),[8] including people's perceived risk of developing a particular health condition, perceived severity of the health condition, and the perceived benefits, weighed against the costs, of an associated health behaviour. The HBM has been applied to a variety of health behaviours, such as breast cancer screening practices.[5 6 9] Patients' beliefs about the personal costs of medical clinic visits have also been shown to affect appointment keeping rates.[10]

The Children's Hospital of Eastern Ontario (CHEO) genetics clinic provides diagnostic and counselling services to patients of all ages, including routine advanced maternal age (AMA) counselling, personal or family history of known genetic disease, and the assessment of subjects whose condition is of unknown cause. At a time when clinical demands on our programme are increasing, we became concerned about the negative impact of no shows on our ability to deliver efficient and timely services. On that basis we undertook a study in order to determine the approximate rates of appointment cancellations and no shows at different Canadian genetics clinics, and to identify factors that may be associated with missing clinic appointments. It was hoped that some associated variables might be amenable to modification and lead to improved attendance rates.

Twenty genetics clinics across Canada responded to a survey regarding the frequency of broken appointments (no shows and cancellations). The centres provide genetic services free of charge as part of their respective provincial health services. The non-attendance rate at the CHEO genetics clinic was also determined. The clinics were separated into three groups according to number of patients seen per year; eight clinics had fewer than 500 patient visits per year (small), eight saw between 500 and 2000 patients per year (medium), and five clinics saw more than 2000 patients per year (large). Representatives of each genetics clinic, usually a medical geneticist or clinic administrator, completed a single page postal questionnaire designed to assess their estimated rates of missed appointments, the extent to which they considered these rates to be a problem, and the strategies they used to reduce non-attendance.

The CHEO genetics clinic operates according to the following pre-appointment procedure. Patients are referred to the clinic by their physician. The clinic receptionist schedules the appointment and, for non-AMA cases, sends the patient a family history questionnaire and consent form for release of medical information. Before the clinic appointment, the patient's case is reviewed with relevant documents and, for non-AMA patients, a family pedigree is drawn. Non-AMA patients are contacted by telephone 24-48 hours before the scheduled visit in order to confirm their attendance (AMA patients do not receive a reminder telephone call). At all stages, patients are asked to cancel if they do not plan to attend their clinic visit.

Data were collected by telephone from two groups of patients originally scheduled for clinic between 1 February 1998 and 30 April 1999: 75 who attended their appointments at the CHEO genetics clinic and 62 who either did not show up for their appointments or who cancelled with less than 12 hours notice. It should be noted that late cancellations (less than 12 hours notice) were counted as no shows because the ensuing consequences were considered to be equivalent. The other surveyed genetics clinics provided separate rates for cancellations in general and for "pure no shows".

A parent was interviewed if the index patient was under 18 years of age. All participants (total n=137) were English or French speaking and lived in the Ottawa-Carleton regional catchment area of approximately 1 million.

Two slightly different versions of the survey instrument were used, one for each group of participants. The instrument was developed by the authors to assess information in four main content areas: (1) demographics (age, marital status, children, education, family income, language spoken at home); (2) referral and genetic service information (reason for referral, the degree to which patients understood these reasons, the quality of explanations provided by referring physicians regarding these reasons, whether or not patients were referred at their own request, and the degree to which referring physicians recommended the genetics appointment); (3) environmental factors (transport, distance from home to the clinic, and arrangements for child care and taking time off work); and