Abstracts of the Annual Scientific Meeting of the Association of Clinical Cytogeneticists held on 30 June to 2 July 1987 at Wills Hall, University of Bristol

A case of diploid/triploid chimerism
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The finding of both diploid and triploid cell lines has been reported previously in patients with hypomelanosis of Ito. In such subjects, this apparent 'mosaicism' is thought to be confined to the skin since their blood lymphocytes have a normal diploid karyotype. Study of cytogenetic and DNA polymorphisms has revealed no evidence of chimerism in such cases to date. A case is presented in which there are pigmentedary changes to the skin along the lines of Blaschko with a diploid blood lymphocyte karyotype and diploid/triploid skin. In view of there being a sex mismatch between the skin cell lines (46,XX/69,XXY), it is postulated that this case represents a true chimera. The fact that this child also has an ovotesticular further suggests that the chimerism may be present in other tissues. Possible mechanisms for the origin of the chimera are discussed. The potential benefit of cytogenetic study in patients with skin pigmentation changes is examined with regard to differentiating between monogenic disorders and cases of chimera for the purposes of genetic counselling.

Mosaic partial monosomy 15q from a 15;16 translocation
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Amniotic cell culture from a 38 year old woman showed a de novo mosaic 46,XX,t(15;16)(q14;p13-3)/45,XX,−15,−16,+der(16)(t(15;16)(q14;p13-3). The der(15) chromosome, comprising 15pter→q14, was missing in approximately 60% of the cells. The mosaicism might have been an artefact and fetal blood sampling and high resolution ultrasound scanning was recommended. The parents were advised of the relative risks and opted for a TOP without further investigation. The fetus showed minor dysmorphic features and culture of fetal blood confirmed the mosaic karyotype. Interpretation and counselling is difficult in this type of case and parents should be fully advised of relative risks before being subjected to the delays and trauma of extended investigations.

Anthropometric studies in the fragile X syndrome
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A series of 46 anthropometric measurements was obtained from 365 ambulatory adult mentally retarded males resident in an institution in Northumberland. They comprised 279 with a normal karyotype, 29 with the fragile X, and 57 with other assorted abnormal karyotypes (excluding Down's syndrome). The males with the normal and assorted abnormal karyotypes were pooled, since their measurements were not significantly different, and compared with the fragile X males. The two groups had a similar physique, but the fragile X males were significantly larger overall. However, the hands and feet of the fragile X males were proportionally longer and wider; their heads were of greater width and circumference and they had longer, thinner faces with the extra length caused by an elongation of the jaw. These quantitative findings supported the presence of physical characteristics previously described after quantitative observations. All of the fragile X males were identified correctly by discriminant analysis on the basis of 20 measurements.

A fertile mule and hinny in China
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Anecdotal reports of fertility in female mules (mare × jack donkey) and hinnies (jenny donkey × stallion) have appeared in published reports over the years, but scientists have generally regarded them with scepticism. The fact that these hybrids can come into oestrous and ovulate makes fertility a possibility, given that opportunity for mating arises. In China, where mules are bred extensively for work on the farms, a fertile female mule and a fertile female hinny have now been verified by chromosomal investigation. Each had mated with a donkey and produced a female foal. The foals show unique hybrid karyotypes different from the mule or hinny and different from each other.
The use of cytogenetic techniques and Y specific DNA probes to investigate a suspected (Y;14) translocation product

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Cytogenetic investigation of a woman following repeated miscarriages revealed a 14q+ marker chromosome. Cytogenetic techniques, including anaphase studies, C banding, and DA-DAPI staining suggested the chromosome was the product of a (Y;14) translocation, but did not permit the assignment of a Y chromosome breakpoint. Family studies have demonstrated unbalanced karyotypes involving this product in five members of three generations, the only adult male carrier being referred independently as a result of his infertility. His sperm exhibited 100% abnormal forms and no motility. DNA studies using a panel of Y specific probes have been used to demonstrate the extent of Y chromosome involvement.

Analysis of the origin of Turner's syndrome using DNA probes

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Thirteen nuclear families with a child or fetus with Turner's syndrome were studied using DNA restriction fragment length polymorphisms. The karyotypes of the probands were as follows: five had 45,X karyotype, four were mosaics with 45,X/46,Xr(X), two were mosaics 45,X/46,Xi(Xq), one was mosaic 45,X/46,XX, and one was 46,Xi(Xq). Of the 45,X probands, two were children and there were three terminations following prenatal diagnosis. Using the DNA probe St14-1 (DX552) which maps to Xq28, parental origin of non-disjunction could be determined in 10 cases, and in the 45,X cases loss of the paternal homologue was more frequent than the loss of the maternal X. By simultaneous use of further DNA probes from the proximal long arm and Xp it was possible to determine the timing and origin of mosaicism and iso-chromosome formation.

Localisation of chromosome breakpoints in ataxia telangiec-tasia lymphocytes using in situ hybridisation

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Various chromosomal translocations have been described in peripheral lymphocytes from patients with ataxia telangiectasia often involving the sites of the immuno-globulin superfamily genes (Aurias et al, Hum Genet 1986;72:210). Clones of cells with some of these translocations may appear in the blood, most being very small. We have studied two large non-leukaemic clones (70% of T cells) with translocations t(14;14)(q11;q32) and t(X;14) (q28;q11) respectively. Using in situ hybridisation of probes on the t(14;14) clone we have shown that the 14q32 breakpoint lies outside the IgH locus and proximal to it with respect to the centromere. The 14q11 breakpoint in the homologous 14 splits the T cell receptor α chain locus with the constant region being translocated to the 14q+ chromosome leaving the variable region on the 14q- chromosome (Kennaugh et al, Hum Genet 1986;73:254, and unpublished results). In the t(X;14) the constant region of the α chain locus is translocated to Xq28, and although a reciprocal translocation is likely from X to 14, the breakpoint appears distal to the site of the RFLPs St14 and to the G6PD gene. Both clones therefore appear to involve translocation of a single T cell receptor gene to the site of an unidentified gene. Involvement of the α chain gene may be required for successful non-malignant T cell proliferation and further genetic change is required for transformation to full malignancy.

Molecular analysis of the fragile X syndrome

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The fragile X syndrome is associated with a fragile site at Xq27-3 and is a disease that affects approximately 1 in 1100 males. The disorder shows several unusual features in that there is a high rate of expression in female heterozygotes and normal males have been found to transmit the mutation. A full understanding of the molecular basis of these observations awaits the isolation of DNA sequences within the region of the fragile site. The DNA probes localised to Xq27→Xqter that bridge the fragile site have been used to construct a genetic map of the region. It is evident that the genetic distances are quite large compared to the physical distances and that genetic heterogeneity exists between affected families. Long range restriction mapping by pulsed field gel electrophoresis analysis is invaluable for the localisation of new genes and the probing of the fragile site region. In particular, clusters of sites for rarely cutting restriction enzymes can be identified which represent good candidates for HTF islands. HTF islands are associated with expressed sequences in the mammalian genome and therefore represent a method by which we can identify candidate genes for this particular disorder.

Biotin labelled in situ hybridisation maps 1 kb single copy gene

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A method is described for the fine mapping of single copy genes using biotin labelled DNA probes and an alkaline phosphatase detection system. The technique may be used on slides which have been previously trypsin G banded and mounted, permitting the use of routine clinical cytogenetic material, possibly archival. Other advantages over radioactive methods include safety, low background, accuracy, and speed, development of label occurring within one to four hours as opposed to two to three weeks for 3H labelled probes. The higher resolution obtained with this method has allowed a number of single copy genes, with insert sizes as small as 1 kb, to be mapped with precision.
The technique would be of use in the identification of marker or supernumerary chromosomes and the appearance of label in interphase nuclei may allow detection of sex or exclusion of trisomy in non-dividing prenatal samples.

The effect of methotrexate on the quantity and quality of metaphases in ANLL bone marrow cultures

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Analysis of bone marrow cultures from leukaemic patients is often impaired by chromosomal contraction and low mitotic index (MI). The well known cytogenetic technique of methotrexate (MTX) block/thymidine release synchronisation, considered to improve mitotic yield and chromosome length, has been examined in detail. Acute non-lymphocytic leukaemic (ANLL) bone marrows were cultured, according to the standard technique, and incubated +/− MTX before release. No significant difference was detectable in either MI or chromosome length. Cell division in the presence of MTX was examined by harvesting after 17 hours with MTX without releasing and no significant block was evident. Pretreatment incubation times and duration of colcemid exposure were also examined. No significant change in cell division or improvement in chromosome quality attributable to MTX was detected, and its suitability for bone marrow synchronisation is questionable. Results indicating improved chromosome length after ethidium bromide treatment will be discussed.

Acute megakaryoblastic leukaemia in childhood. The role of chromosome 21

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A case of acute megakaryoblastic leukaemia in a two year old girl with Down’s syndrome is presented. This rarely reported malignancy has been described more frequently in children with Down’s syndrome. Normal children with this leukaemia frequently have acquired trisomy 21. The leukaemic cells in the present patient have a previously unreported chromosomal change interpreted as dic(5;7)(p12;p11.1).

How can chromosome 9 or 22 involvement be hidden in aberrant translocations in chronic myelogenous leukaemia (CML)

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Besides two personal cases (t(4;22)(p16;q11) and t(12;22) (p13;q11)), we collected 78 such translocations (t(M;22) and t(M;9)) involving chromosome 9 or 22 and an extra chromosome (M and/or M’). The breakpoint on M was defined as telomeric (T) or not (NT). In a t(M;22) T, number 9 involvement could be masked if, in a circular translocation t(M;9;22), the 9 telomere is replaced by a telomere of M the same size and grey level. Abl would be translocated next to Bcr as usual in CML. In a t(M;22)/NT, a short gene sequence from chromosome 9 would seem of normal size. In a t(M,9)/NT, a short gene sequence including Bcr would be translocated onto M before 9 telomere including Abl. These last two configurations have already been ascertained by in situ hybridisation. In a CML Ph−, only a short gene sequence including Abl would be translocated next to Bcr. Finally, six cases of t(M;9), t(M’;22) have been published, five of them with a telomeric breakpoint on M’, M’ telomere could be translocated onto M without being detectable, a part of M onto 9 as detected, 9 telomere onto 22 as usual in CML, and 22q12−qtter onto M’ as detected, in a circular translocation t(M;9;22,M’). Similarly, the usual t(9;22) might occasionally be a t(M;9;22) where M involvement is hidden.

Myelodysplastic syndromes. Clonal origin and evolution

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The myelodysplastic syndromes (MDS) constitute a heterogeneous group of clonal disorders, arising from a multipotent haemopoietic progenitor, which share a leukaemic propensity, 30% of cases culminating in acute myeloid leukaemia (AML). Non-random chromosomal abnormalities are documented in 30 to 79% of cases and karyotypic evolution is usually associated with a clinical progression of the disease. Their pathogenesis probably entails multistep, phenotypic progression being determined by either expansion or evolution of the abnormal clone. The clonal origin of certain cases of de novo AML is analogous to that of MDS and evidence that they share a common pathogenesis and distinct biological characteristics is beginning to emerge. However, difficulties in early diagnosis remain, since morphological abnormalities may be subtle and karyotypic abnormalities may be absent. In view of this, alternative methods of diagnosis of clonal expansion which would complement chromosomal analysis are being attempted. Preliminary results of the use of sex linked DNA polymorphism at the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus are encouraging. The application of techniques currently in use in molecular biology may help us understand the pathogenesis of this disease which has been called a human model for the study of leukaemias.

Cytogenetic and DNA polymorphism studies in leukaemia patients having bone marrow transplants

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Christie Hospital and Holt Radium Institute, Manchester. 14, 2023 by guest. Protected by

The value of cytogenetic and DNA polymorphisms as early indicators of engraftment and relapse after bone marrow transplantation is being assessed. Leukaemic cells are often marked by chromosome abnormalities, which usually reappear in relapse. In the absence of chromosome abnormalities, the sex chromosome difference is the most widely used technique to document engraftment. When
Counselling and segregation

In the absence of striking chromosomal variations, restriction fragment length polymorphisms (RFLPs), which indicate variation in the primary DNA sequence, may be used. A number of transplant cases have been studied using cytogenetic and DNA techniques. The findings will be presented and the relative values of the two procedures in monitoring transplantation will be discussed.

Interstitial deletion of chromosome 13 in three cases with retinoblastoma: further localisation of the Rb1 gene

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Retinoblastoma, a childhood tumour, occurs with a frequency of about 1 in 10 to 20 000. The majority are sporadic but about 5% have an autosomal dominant mode of inheritance with 90% penetrance. Some 5 to 10% of sporadic cases have a cytogenetic imbalance, which is usually the result of an interstitial deletion. We report three further cases of interstitial deletions of chromosome 13, del(13)(q13-2q22), del(13)(q13-3q22), and del(13)(q14-3q22), associated with retinoblastoma. The tumour was unilateral in two cases and bilateral in one. The levels of the linked enzyme esterase D were 57%, 54%, and 64% respectively. One deletion case was ascertained through the retinoblastoma but the other two were ascertained through failure to thrive or dysmorphism or developmental delay. The cytogenetic findings alerted the clinicians to the possibility of a tumour in one child and early treatment avoided the resort to enucleation. Dysmorphic features were observed in all three children. The proximal breakpoint within 13q14-3 appears to exclude the genes for retinoblastoma and esterase D from 13q14-1 and 13q14-2.

Are double translocations double trouble?

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Double translocation heterozygotes are rare, but need not pose more of a counselling problem than single reciprocal translocations. Nine cases of double translocations will be briefly presented and used to illustrate possible counselling guidelines for the assessment of risk of producing a liveborn abnormal child. These suggested guidelines are not based on theoretical considerations or segregation patterns, but are extrapolated from what is known empirically about segregation patterns in carriers with single reciprocal translocations. An assumption is made that there is no interference with the independent assortment of two separate exchanges, unless a common participating chromosome is involved and the possibility of an interchromosomal effect is not taken into consideration.

Prenatal diagnosis of a pure trisomy 14/18 mosaic

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Trisomy 18 was diagnosed in the fetus of a 40 year old mother of five following amniocentesis at 17 weeks' gestation. The phenotype of the fetus after TOP was consistent with this diagnosis. However, cultures of skin and chorion produced two cell lines with trisomy 14 predominant over trisomy 18 in both skin (82%) and chorion (65%). Subsequent re-examination of preparations from the original amniotic cell culture confirmed trisomy 14 as a minor cell line only (4%). The possible origin of this unusual case is discussed.

Prenatal diagnosis of fragile X syndrome by placental biopsy

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Two out of eight male fetuses (gestation 19 to 20 weeks) at risk of fragile X linked mental retardation (Martin-Bell syndrome) were diagnosed as affected after cordocentesis and subsequent fetal blood culture (4% and 21% of cells with fra(X) respectively). In both cases fra(X) was also demonstrated in placental biopsy cultures (PBC) (0-4% and 4% of cells respectively). Fra(X) was demonstrated using methotrexate, 5-fluorodeoxyuridine (FUDR), and excess thymidine. Fra(X) expression with FUDR was not enhanced by caffeine or 5-methoxybenzamide. The use of caffeine may lead to ambiguous results.

Long range restriction mapping of human chromosomes

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Since molecular cloning and sequencing operates on DNA molecules of up to a few kilobases length, and since cytogenetics and family linkage studies do not provide resolution below the million base pair level, there has existed a 'resolution gap' in the range of techniques available to human geneticists. It is now possible to fill this gap using various forms of pulsed gel electrophoresis, which are capable of separating DNA in the size range 50 milobases to 10 megabases. By using these techniques in conjunction with restriction enzymes that cut human DNA very infrequently, long range maps of chromosomal regions and even whole chromosomes can be constructed, cytogenetically invisible DNA rearrangements can be detected, and strategies for isolating disease genes can be envisaged. Some preliminary data involving human chromosome 19 have been obtained.

The effect of deletions on carrier status determination in DMD

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The Wessex Regional Cytogenetics Unit is compiling a DNA register of families within the region with a history of
Further coding sequence at CVS and carrier mutation. The linkage islands signalling coding sequences, St of made the understanding of bases, material, and research have been isolated which probes can be attempted for the candidate, CROLLA, ZAHED, MCGUIRE, CROLLA, Z DOCHERTY, C FEAR, and M BOBROW Paediatric Research Unit, Guy's Hospital, London.

For the past year, our group has been working on CVS material, providing a diagnostic service mostly for the Guy's MRC CVS-amnio trial, while efforts have been made to improve the cytogenetic techniques and to understand the biology of this tissue. Diagnostic experience. We have analysed 330 cases using direct (DP) and culture (CP) protocols. Direct preparations were suitable for chromosome count, sexing, and GTG banding. Analysis of metaphases up to the 300 band stage was possible in 70% of the cases. In our experience we have encountered four situations which had to be carefully evaluated: mosaic trisomy 7 detected only in DP and not confirmed in CP, analysis of two familial rearrangements ((t(4;5)(q35;q33),inv or iso(13) which were only possible in CP, and fine rearrangement of chromosome 8p detected in CP but missed in DP. Research experience. Direct, semi-direct, and culture protocols have been adapted to CVS material in an effort to obtain good preparations in short times. Explant cultures have been successfully harvested within 11 days. To accelerate the analysis of DP, automatic scanning has been evaluated using the Magiscan and a laboratory research precursor of Metafil. For a complete cytogenetic analysis of DP standard banding protocols have been modified, namely QFO, RFG, CBG, DAPI and NOR. Cell cycle studies have been performed using BUdR incorporation, which have allowed us to establish protocols for first trimester prenatal diagnosis of chromosome instability disorders.

Can the 'chorionscope' help in providing a diagnostic CVS service for a distant district hospital
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Use of the chorionscope permits direct visualisation of the chorion biopsy, thus reducing the dependence of the obstetrician on high resolution ultrasound equipment and dispensing with the need for immediate evaluation of the sample under the dissecting microscope. This approach, in conjunction with rapid rail transport of the specimen to the laboratory, is currently under evaluation and it is hoped that the provision of a diagnostic CVS service by the District Hospital at a distance from the cytogenetics laboratory will eventually become possible.

Pseudomosaicism in chorionic villi
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The problems of distinguishing true from false mosaicism in amniotic fluid cells are well known. Similar difficulties are sometimes encountered in cells from chorionic villi. The case is described here where all cells from a semi-direct (3 hour) culture had a complement of 48,XX,+18,+20. In the long term culture (eight days), however, only 45% of the cells were abnormal. True mosaics for +18 are rare and should be investigated further. In fact, in this case a fetal blood sample showed a normal 46,XX in over 100 cells examined. The pregnancy is continuing.

Evaluation of sampling procedures in cytogenetics: and exercise in quality control
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Confirmation of the coexistence of two cell lines (mosaicism or chimeraism) in the same subject is a frequent problem for cytogeneticists who should rely for this task on sound sampling procedures. These are often dictated by staff shortages rather than by acceptable statistical practice. Without making rigid suggestions, we propose a simple way to deal with this problem in its various forms. We have plotted sample size against frequency of second cell line to obtain a given probability of detecting 0, 1, 2, or 3 cells of the less abundant line. This suggests an iterative procedure to deal with rare mosaicism detectable in blood cells and several situations will be discussed. A similar method is described to detect genuine fetal mosaicism as it would appear in amniotic fluids harvested in situ or by the flash technique, to distinguish it from pseudomosaicism. For the diagnosis of fragile X, we show the power of sampling plans 50(0-1), 50(1-2), 100(0-1), 100(1-2), 200(0-1), and 200(1-2) as defined in British Standard BS 6001, 1972.