4.003  Improving the molecular diagnosis for facioscapulohumeral muscular dystrophy (FSHD).

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FSHD provides a direct method for the diagnosis of the majority of FSHD patients. However, this diagnosis has been hampered by the presence of locus heterogeneity, the detection of non-4q35 fragments, and the occurrence of small fragments in the normal population which has reduced the overall test specificity to 76% when using 28kb as the cut-off point. A recently described method, involving the use of two restriction enzymes EcoRI and BglI, excludes the 10qter alleles and shortens the 4q35 fragments by 3kb. While improved diagnostic accuracy is predicted with this method, to date its use has been reported on 19 familial and 4 sporadic FSHD patients with no data yet available from normal controls. Therefore validation of this observation in a large panel of FSHD and control subjects is warranted. Among 100 unrelated FSHD patients, in whom a small EcoRI fragment had been previously identified, a 3 kb reduction in fragment size following the double digest was observed in all cases, providing confirmatory support for the earlier report. Among 308 chromosomes from control subjects with no clinical signs or family history suggestive of an increased risk of FSHD, the smallest fragment observed was 38 kb. Thus, at the 35kb cut-off point, the exclusion of 10qter alleles among these normal controls raises specificity to 100% and suggests that the diagnostic cut-off could be modified, allowing increased test sensitivity with minimal reduction in specificity. Using this new information, we have reassessed six large 4q35-linked families who had previously ambiguous results due to the presence of 3 or 4 alleles from the two separate loci. The new protocol allowed successful identification of the 4q35-specific alleles in all 6 families. Our results clearly demonstrate that the double digest method will improve significantly the accuracy and informativeness of the molecular diagnosis of FSHD. We are now using this method for prenatal and presymptomatic diagnosis.

SECTION 4  
Prenatal genetics/diagnosis and cytogenetics

4.001  Duplication and deletion of 5q22q23 in several offspring resulting from an inter chromosomal insertion in the parent.

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A familial interchromosomal insertion involving chromosomes 5 and 10, 46;XY,ins (10;5)(q25;q22q23) was found in the father after the birth of a dysmorphic child. Following the birth of the child with a deletion of 5q22q23, 46;XY, der (5) ins (10;5) (q25;q22q23)pat, three further fetuses with the same rearrangement have been detected prenatally. In one pregnancy prenatal diagnosis was refused and no scan anomalies were evident. At birth the child had no dysmorphic features but in view of the family history, cytogenetic studies were done and duplication of 5q22q23 was revealed, 46;XY,der (10) ins (10;5)(q25;q22q23). The fetuses with a deletion of 5q22q23 had a variable phenotype but talipes and arthrogryposis were a common feature. Interestingly the child with a duplication of 5q22q23 is clinically normal at the age of nine months. Further discussion of the individual cases will be presented.


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Robertsonian translocation carriers can usually achieve normal pregnancies with the help of prenatal diagnosis where necessary, occasional couples present with a history of infertility. Such couples are being referred for preimplantation genetic diagnosis (PGD). We have been carrying out PGD for two such couples: 1.45,XY, (der13;14)(q10;q10) ; 2. 45,XX, (der13;21)(q10;q10). The first couple have undergone 2 cycles of IVF, the second 1 cycle. In total 12 of 24 embryos were suitable for biopsy and possible transfer; two cells were tested from each on day 3 post fertilisation (8-10 cells) by triple colour FISH using locus-specific YAC probes. Three only were normal for the tested chromosomes and hence transferred. The remaining 9 biopsied embryos together with the 12 not biopsied were subjected to full analysis of individual chromosomes. Of the 21, 3 were monosomic, 1 nullisomic, 1 trisomic and tetrasomic, 2 monosomic and mosaic and 12 were so abnormal that they were classed as 'chaotic'. (chromosome constitution varying randomly from cell to cell); 2 embryos arrested at 2 and 4 cells were apparently normal. In addition to aneuploid segregations, the extremely high frequency of mosaicism and chaotic divisions could explain the infertility in these couples.
4.004  
Prenatal diagnosis of trisomy 2 mosaicism  
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Very few reports of trisomy 2 mosaicism have been detected at amniocentesis. One was confirmed at birth in a dysmorphic child, subsequently lost to follow up. Another resulted in a developmentally normal, non-dysmorphic child with bronchopulmonary dysplasia, hypothyroidism and growth retardation who had maternal disomy for chromosome 2. This paucity of information hinders counselling in pregnancy. We present a case of trisomy 2 mosaicism (47,XY, +2/46,XY) detected at amniocentesis; trisomic cells were present in 51/96 (57%) of cells from three cultures. The mother was a 31 year old G3P1, given a composite Down Syndrome risk of 1:90. Ultrasound scan showed dilatation of the left pelvicalycal system, right talipes equinovarus and a pericardial effusion. After counselling the parents opted for termination of pregnancy. Subsequent examination of the fetus showed asymmetry of the nose, ears and palpebral fissures, notched alveolar margins and prominent naso-labial and infra-orbital creases. Additionally, radiographs revealed a left thirteenth rib and post mortem demonstrated an absent gall bladder. Trisomy 2 mosaicism was confirmed in fetal skin fibroblasts.

4.005  
A case with two de novo rearrangements with multiple breakpoints involving chromosomes 11 and 15, detected prenatally  
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We describe an unusual prenatal case with a complex karyotype with two apparently unrelated de novo rearrangements involving chromosomes 11 and 15, with a minimum of five chromosomal breakpoints. An amniotic fluid sample from a 29 year old patient at 17 weeks gestation showed a strawberry shaped head with bilateral ventriculomegaly and renal problems on ultrasound scan. Cytogenetic analysis revealed a male karyotype with one abnormal chromosome 11 and a rearrangement of one chromosome 15. The abnormal 11 appeared to result from a complex rearrangement involving a pericentric inversion and a deletion of material from the short arm. The abnormal 15 appeared to have a paracentric inversion in the long arm, but without obvious loss of genetic material. Parental chromosome studies were normal indicating that the rearrangements observed in the fetus were de novo. Further characterisation of the abnormal chromosomes was done using chromosome 11 and 15 paints and probes WTI (11p13), MLL(11q23) and SNRPN (15q11-q13). The karyotype of the fetus was finally described as: 46,XY,del(11)(p13.1q21.3)inv(11)(p13q22.1).inv(15)(q13q21.3).inv(15)(q13q22.1).  

The chromosome rearrangements observed appear to involve a minimum of five breakpoints with a deletion of material from chromosome 11 and therefore would be expected to have significant phenotypic consequences. This pregnancy is currently ongoing.
4.007
Chromosome analysis and detection of mosaic karyotypes in 6-8 day old human blastocysts
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Confined placental mosaicism (CPM), chromosome abnormalities restricted to the placenta, reflects a combination of post-fertilization mitotic errors in normal conceptions, and correction of meiotic errors (reduction to disomy) in trisomic conceptions, with compartmentalisation of the abnormal cells into either or both the cytotrophoblast and extraembryonic mesoderm cell lineages. To produce the abnormal cell distributions seen at the end of the first trimester, the key mitotic non-disjunctional or anaphase lag events must occur within the early cell divisions post-fertilization, either before or soon after blastocyst formation. Current techniques for investigation of cytogenetic anomalies at this stage of development, produce only limited numbers of poor quality metaphases, often without banding and unsuitable for useful detection of mosaicism. FISH technology can be used to evaluate mosaicism in interphase cells, but can only produce a partial analysis restricted to the probe combinations which can be processed simultaneously. We describe a technique for preparation of good quality chromosome preparations from thymidine synchronized, 6-8 day old, spare IVF blastocysts, by disaggregation into single cells using 60% acetic acid. This technique is compatible with all standard cytogenetic and molecular cytogenetic procedures, and will enable investigation of the origins of CPM at this stage of human development.

4.008
Prenatal diagnosis of mosaic trisomy 8 with detailed investigations of the extent and origins of the chromosomes 8.
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We describe a detailed case of mosaic trisomy 8 detected prenatally following CVS performed for renal abnormalities detected on ultrasound scan. Chromosome analysis of direct preparations showed a 46,XY karyotype. Analysis of long term cultures showed an abnormal 47,XY,+8 karyotype (50 cells). Cultured cells from a fetal urine sample obtained at the same time as the CVS showed a mosaic trisomy 8 karyotype (3/53 cells +8). At 20 weeks the pregnancy was terminated. Post mortem examination showed a male fetus with joint contractures, a vertebral defect, hydronephrosis, hydrourter and bladder dilatation. Trisomy 8 cells were confirmed in post mortem skin and muscle samples, (4/30 and 4/50 cells respectively). There was no evidence of trisomy 8 cells in cardiac blood cultures (100 cells). FISH studies performed on cytotrophoblast preparations and corresponding long term placental cultures confirmed the presence of trisomy 8 cells at all sites investigated. Analysis of kidney cells showed a low level of trisomy 8 mosaicism. Microsatellite PCR analysis of fetal and parental DNA showed that the trisomy 8 cell line arose following a post-fertilisation mitotic error. This is only the second case of prenatal detection of true trisomy 8 mosaicism, with detailed cytogenetic analysis of extraembryonic tissues.

4.009
The prenatal diagnosis of epidermolysis bullosa simplex
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Epidermolysis bullosa simplex (EBS) is a skin fragility syndrome characterised by rupture of the basal keratinocytes of the epidermis in response to mild physical trauma. The severest form of EBS, Dowling Meara (EBS-DM), is an autosomal dominant condition associated with widespread blistering which appears at, or shortly after birth. Mutations associated with EBS-DM occur in highly conserved regions of keratin 5 and 14. In particular, alterations of codon 125 of keratin 14 account for approximately 60% of mutations. This has facilitated the development of a screening strategy whereby these conserved regions are amplified from genomic DNA and the products sequenced directly. Two affected families were screened and the causative mutation identified in both as an Arg to Cys alteration of the common codon 125 site. In one family the condition was shown to be familial, the mother and fetus lacking the mutation. In contrast, the other family represented a sporadic case, neither parent possessing the causative mutation. Until now, prenatal diagnosis has been performed by fetal skin biopsy at 18 weeks gestation. However this current approach allows prenatal diagnosis within the first trimester of pregnancy for the majority of EBS-DM cases.

4.010
Incidence of 22q11 deletion and karyotype abnormalities in fetuses with cardiac abnormalities detected prenatally.
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Microdeletion within chromosome 22q11 region is associated with a wide range of cardiac defects; however, the incidence of 22q11 deletion in the cardiac population overall has not been determined. We have undertaken a one year prospective study to establish the incidence of 22q11 deletion in fetuses of all mothers referred to the regional fetal cardiac department who have a cardiac abnormality in their fetus. In all patients fetal karyotype and FISH analysis was offered irrespective of the nature of the cardiac defect. Of the 222 cases referred results are available on 128. 2 fetuses were found to have a 22q11 deletion and 24 fetuses had an abnormal karyotype. These included 2, 3, 3, and 12 cases of trisomy 9, 13, 18 and 21 respectively. There was 1 case of 45,X and 1 case arose due to an unbalanced translocation where the mother had a previously undeleted balanced translocation. The high proportion of karyotype abnormalities (19%) detected suggests that all fetuses with cardiac defects should be karyotyped. As 2 of 104 with normal karyotype had 22q11 deletion detected by FISH we suggest that this test should be offered for all fetuses with a cardiac abnormality where a normal karyotype has been documented.