Recently recognized chromosomal defects of clinical importance

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Summary: We review those conditions which have recently been recognized to be associated with small, sometimes difficult to detect, chromosomal abnormalities. These include the Prader-Willi syndrome and X-linked mental retardation.

Introduction

Edwards, Harnden, Cameron, Goss and Wolff (1960) described a new trisomic syndrome – which they attributed to a triplication of either chromosome 17 or 18 (later proven to be 18). Wolff, in that historic article, is the subject of this Festschrift and this review is dedicated to him in respect of his lifelong contribution to so many aspects of paediatrics. Only a few years before that publication, Lejeune (1959) had shown that Down's syndrome was linked to the presence of an extra chromosome – designated a G-group chromosome, and now as chromosome 21. Trisomy 18 was followed by 13 (Patau *et al.*, 1960, the next article in the same journal), and then a whole host of partial trisomies and deletions were described, some with recognizable phenotypes but others showing great diversity.

There was a major advance in 1970 with the development of banding by Caspersson *et al.* This led to easy recognition of individual chromosomes and an improvement in the detection of small deletions; 350 bands could be seen by conventional G-banding but it was obvious that there might be small deletions, too small to be visualized by routine methods. This was predicted by clinicians who had long suspected that amongst the dysmorphic patients with multiple malformation syndromes, including defects of the heart and mental retardation, known not to be inherited in a simple Mendelian fashion, there would be undetectable deletions.

In 1976, Yunis introduced the term 'high resolution' chromosome banding to describe a technique pioneered in his laboratory to advance visualization from the 350 band stage to 400 and beyond that to an 850 stage. Resolution has, even under exceptional circum-

stances, delineated 1000 bands. The techniques are expensive and time consuming but their greatest contribution might be to identify significant changes in the genome which can, retrospectively, be seen by conventional methods once an area of possible change is recognized.

Improvement in technology has to some extent blurred the clinical distinction between single gene defects and chromosomal abnormalities. Chromosomal abnormalities are generally regarded as those that can be observed by microscopy whereas single gene disorders are those with normal chromosomes in which the pattern of inheritance conforms to the well worked out Mendelian ratios. It is now realized that what might appear to be simple Mendelian inheritance can be found to be chromosomal in origin. Examples are given.

Autosomal deletions

Aniridia

In aniridia, the iris is rudimentary and the remnant may block the angle to cause glaucoma. The anterior chamber is shallow, resulting in contact between the cornea and lens. Vision is poor. The condition is known to be dominantly inherited in a number of families in which the defect is transmitted vertically from generation to generation. In this form, the chromosomal analysis is normal. However, there is also a known association of aniridia with Wilms' tumour (nephroblastoma) and in a number of these patients a deletion in the 11p 13 region has been found (Francke *et al.*, 1977) – that is, in the short arm of chromosome 11 near the centromere. There is also a

suggestion that an 11p deletion can cause Wilms' tumour alone, especially when hemihypertrophy is present. In addition, there have been reports of patients with 11p 13 deletions with aniridia alone (Ferrell & Riccardi, 1981). It is here that high resolution banding becomes important. Nakagome and colleagues in 1984 looked retrospectively at 5 cases of aniridia/Wilms' tumour and all showed the characteristic change. However, it is not always easy to find – in one of these cases a small deletion was found in only half of the examined cells.

What seems to be emerging is a relationship between overlapping syndromes, dependent on the extent of the lesion. Perhaps just a single base change or tiny deletion at a particular locus in the genomic DNA can result in dominantly inherited aniridia. A slightly larger change, i.e. a small deletion in band p13, involves a tendency to develop Wilms' tumour or aniridia and if the deletion involves both regions then Wilms' tumour and aniridia might occur together.

A similar development has occurred in our understanding of retinoblastoma – although the situation is slightly more complicated.

Retinoblastoma

There is a parallel between the mechanism of development of Wilms' tumour and retinoblastoma (see below). Although most Wilms' tumours are sporadic and vertical transmission within families is rare, it has been shown (Orkin *et al.*, 1984) that if a deletion occurs on one of the short arms of chromosome 11 at p13, then a second mutational event on the other chromosome 11, at the same place but in a somatic cell, could explain the development of Wilms' tumour in at least some patients.

Retinoblastoma is a tumour of childhood which occurs either unilaterally or bilaterally. Vogel (1979) suggested that 40% were hereditary and that this figure encompasses most of the bilateral tumours and 15–20% of the unilateral retinoblastomas. It is intriguing (see Johnson *et al.*, 1982, for review) to find that 5% of retinoblastomas could be explained by what seemed to be a chromosomal deletion affecting band q14 of chromosome 13. These patients had bilateral tumours and the majority were mentally handicapped, albeit on occasions only mildly. This contrasted with those families in which bilateral retinoblastomas were transmitted from generation to generation – seemingly as an autosomal dominant trait – where intelligence was normal.

The condition, as in aniridia, can be caused by a submicroscopic change first thought to be a single gene defect but now thought to be a small deletion. The same end result occurs when there is a visible deletion in q14 of chromosome 13. However, for the retinoblastoma to occur, another mutation is neces-

sary. This is a somatic mutation – an event which must occur commonly in somatic cell DNA. Two gene changes are therefore necessary and the tumour results from homozygosity at a given locus, i.e. by conventional nomenclature it is recessive. In sporadically occurring tumours, both mutations occur in somatic cells.

It therefore seems that a condition which was thought occasionally to be inherited as a dominant with reduced penetrance might be the result of a small chromosomal deletion – sometimes visible and sometimes not.

Further evidence for a small deletion, even though it cannot be seen, comes from experience with esterase-D. The gene coding for esterase-D has been localized to the same band as that known to be deficient in bilateral retinoblastoma. As each band represents many genes it is likely that these loci are at least closely situated in the same region. Arising from this has been the finding of patients with retinoblastoma, with normal chromosomes, in which the activity of esterase-D has been half normal suggesting that a presumptive deletion in the band q13 is present but is not visible by conventional banding techniques – not even by extended banding.

Prader-Willi syndrome

There are a number of conditions which are clinically recognizable but where the diagnosis rests on history and clinical observation. Special investigations to confirm a clinical impression are seldom helpful. This is a particularly difficult situation to deal with in those dysmorphic syndromes where variability of expression occurs. The Prader-Willi syndrome falls into the category of being easy to diagnose when typical

Figure 1 Prader-Willi syndrome.

(Figure 1) but which can be under- or over-diagnosed. Characteristically there are early feeding difficulties, later (second year of life) hyperphagia and obesity, hypogonadism, microcephaly, almond shaped eyes, a tent-shaped mouth, and small hands and feet, but the syndrome is not always easy to diagnose. Ledbetter *et al.* (1981) suggested that the diagnosis could be confirmed in a proportion of patients by chromosome analysis. An interstitial deletion in the long arm of chromosome 15 involving a breakpoint at 15q13 has been the most constant finding.

What of the half without obvious deletion? In these patients, the deletion might be too small to be seen, or there might be other conditions which mimic Prader-Willi. Prader-Willi has led the way in the realization that it is possible that other known syndromes with multiple congenital malformations might turn out to be caused by a small deletion.

Miller-Dieker syndrome (lissencephaly syndrome)

The diagnosis is often suggested clinically by the presence of vertical wrinkling of the forehead in a microcephalic child but this is not invariably present. Severe retardation, seizures, early hypotonia, occasional congenital heart defects and even polydactyly have all been described. The inheritance was thought to be recessive and four, or possibly five, families were found to have more than one affected sib. However, Dobyns *et al.* (1983) found one patient to have a small deletion in the short arm of chromosome 17. When the four previously reported families were re-examined, either balanced translocations or deletions were detectable in the 17p13 regions and the emphasis has now swung from simple Mendelian inheritance to a chromosomal abnormality and high resolution banding needs to be employed on all patients with clinical features of the lissencephaly syndrome (Dobyns *et al.*, 1984).

Langer-Giedion syndrome or tricho-rhino-phalangeal syndrome

Tricho-rhino-phalangeal syndrome has been divided into two types. Type I is characterized by a thin upper lip, sparse hair, bulbous nose, rather minor phalangeal changes and cone shaped epiphyses. Type II, also called tricho-rhino-phalangeal syndrome with exostosis, differs from type I by the presence of microcephaly and mental retardation, redundant skin, especially in the neck and by bushy or normal, rather than sparse, eyebrows. However, four new patients described by Langer *et al.* (1984) were not reported to be retarded and the distinction between type I and type II is not clearcut. They share in common the striking facial characteristics and the cone shaped epiphyses.

Type I is clearly inherited as an autosomal dominant

condition and three generation families have been reported. The inheritance is less clear for type II (i.e. Langer-Giedion syndrome). There has been a father/daughter pair but nothing more extensive than that. It has, however, been suggested that because of the variability of the condition, the diagnosis in the offspring might have been missed, but this is unlikely.

It was then that a number of patients with type II were found to have a chromosomal abnormality. When reviewed by Langer *et al.* in 1984 there were at least 12 patients with a deletion of 8q involving either 8q23 or 8q24. High resolution banding (Bühler & Malik, 1984), has narrowed down the breakpoint even further and these authors designate the common area to be q24.1. It is also of interest that a presumed type I patient (Hamers *et al.*, 1983) has been found to have the deletion.

Clearly, these advances are leading to a better delineation of syndromes (both 'lumping' and 'splitting' will occur), and an improvement in diagnostic criteria for many syndromes must result.

Other recognizable syndromes

So many of the chromosomal abnormalities are not possible to diagnose clinically. This especially applies to the deletions – the main reason being that microscopically it can never be certain that two patients with a deletion at, say, band q14 of chromosome 11 have indeed lost the same amount of material. There are probably 'hot-spots' where breakage is more likely to occur, hence the similarities of patients with the cri-du-chat, (5p-) syndrome. Where this tends to happen, small deletions can be detected because a shrewd clinician has suggested to the cytogeneticist that the syndrome would fit a specific deletion. The 4p- (Wolf-Hirschhorn syndrome) is a good example. Typically, the children are retarded and severely microcephalic. They have a 'Greek helmet' facial appearance caused by the wide nasal bridge and parallel sides to the nose. This gives the nose a square, flat outline. The upper lip is thin, often cleft, and the philtrum short. Preauricular ear tags might be present. There have now been a number of cases on record where the initial chromosome investigation has been normal and only perseverance by a clinician and a re-examination of the chromosomes has enabled the diagnosis to be confirmed.

Following the same theme, 13q- syndrome might be suggested by small or absent thumbs, and in trisomy 8, very deep palmar and plantar creases occur. In 9p trisomy, the hands are characteristic. The palms are long when compared to the length of the fingers and there is often an unusual little finger which is not only small but in which there is fusion of the two finger creases.

There are many other examples of almost unique

clinical features which could suggest a diagnosis and there have even been attempts to localize certain dysmorphic features to specific sites on the chromosome. Diagnostically, this might not be vitally important – especially if all children with multiple handicap have a karyotype performed – but it might help to localize known syndromes to particular parts of the genome – an activity which is gaining ground amongst molecular geneticists in their endeavours to map the genome and thereby improve our prediction of genetic disease.

Structural abnormalities of the X chromosome – Fragile X syndrome

The most dramatic change in the clinical application of cytogenetics in recent years has been the delineation of the fragile X syndrome, probably the commonest form of mental retardation in boys after Down's syndrome, and by no means an insignificant contribution to moderate mental retardation in females.

The disorder that is characterized by mental retardation and the presence of a fragile site – the appearance of an unstainable gap – near the tip of the long arm of the X chromosome at Xq27-8 has been given various names. The term Martin-Bell syndrome comes from the family described over 40 years ago (Martin & Bell, 1943), in which several mentally retarded descendants have been shown to exhibit fragile sites, plus the associated features of macroorchidism and the characteristic facial appearance (Richards *et al.*, 1981). Some of the names emphasizing the cytogenetic aspects, such as marker X mental retardation (Turner & Jacobs, 1983) have now been superseded by the general term fragile X syndrome. There is some merit in the latter term being used as a general designation, with the name Martin-Bell syndrome limited to those family members exhibiting the full manifestations, for, as will be discussed later, not every man who transmits the condition is clinically affected.

The fragile X syndrome has been the subject of some recent reviews (Turner & Jacobs, 1983; Sutherland, 1985) and the January 1984 issue, vol. 17, no. 1, of the *American Journal of Medical Genetics* reports the proceedings of the first international workshop on the subject plus 29 additional papers on X-linked mental retardation. The medical importance of the fragile X syndrome stems from the fact that it is both a common cause of mental retardation and a model for a class of genetic mutations that are as yet ill defined, but seem to be neither the usual chromosomal abnormality, nor a single gene defect inherited in a regular Mendelian fashion. Overall, a third of female heterozygotes have intellectual impairment (Turner *et al.*, 1980), and there is increasing evidence that what is initially transmitted

within some families is a premutation that causes no phenotypic abnormality, but tends to generate the definitive mutation probably by a recombination event (Pembrey *et al.*, 1985).

Prevalence in patient populations with mental retardation and autism

Indirect estimates of the general population frequency have been derived from studies on defined patient populations and it seems the prevalence is about 1 in 1500–2000 males (Turner & Jacobs, 1983; Sherman *et al.*, 1984; Bunday *et al.*, 1985). Between 1 in 700 and 1 in 1000 females will carry the mutation, a third of whom will have mental impairment. The overall prevalence of the fragile X syndrome in males in institutions for the retarded ranges from 1.6% (Sutherland, 1982) to 6% (Froster-Iskenius *et al.*, 1983), but if known chromosomal and dysmorphic cases are excluded the figure rises to about 7–9% of males. In special schools catering for the moderately retarded the incidence was found to be 3.4% in males and 2.3% in females (Turner *et al.*, 1980; Sutherland, 1985).

Prevalence studies in Sweden found that the fragile X syndrome accounted for 4.5% of boys with mild mental retardation (Blomquist *et al.*, 1983); 7% of boys with uncomplicated severe mental retardation (Blomquist *et al.*, 1982) and 16% of 83 boys with infantile autism (Blomquist *et al.*, 1985).

This last observation is one of a number of reports (Brown *et al.*, 1982; Gillberg, 1983; Levitas *et al.*, 1983) pointing to an association between fragile X syndrome and autism. Blomquist *et al.* (1985) report autistic brothers with 11–18% fragile sites, one of whom had an IQ greater than 85. Despite some negative reports (Venter *et al.*, 1984), accumulated published experience points to a genuine biological cause for the association between fragile X and autism. However, the genetic contribution to infantile autism is itself complex, twin and family studies revealing an association between autism and cognitive disorders often characterized by problems with spoken language (Folstein & Rutter, 1977). What contribution the fragile X syndrome makes to the reported 2–3% sibling recurrence risk for infantile autism (Folstein & Rutter, 1977) still has to be resolved, but it undoubtedly accounts for some sib pairs.

The clinical picture of the Martin-Bell syndrome

Until routine cytogenetic detection of the fragile site became possible (Sutherland, 1977) families with Martin-Bell syndrome (Martin & Bell, 1943; Dunn *et al.*, 1963) were regarded as having non-specific mental retardation with no particular distinguishing physical features. However, once the presence of the fragile X became the definitive diagnostic test it has been

possible to discern a fairly characteristic physical appearance which often allows the experienced clinician to predict the cytogenetic findings. Indeed, increasingly the shoe is on the other foot, with the clinician insisting that the patient has precisely the right combination of physical and behavioural characteristics, but with the cytogeneticist being unable to demonstrate the fragile site on the X. Fishburn *et al.* (1983) studying a cohort of males with X-linked mental retardation found 18 that had the characteristic features of the Martin-Bell syndrome, including macro-orchidism, but in 6 no fragile sites could be demonstrated. Whether or not such cases have a different mutation at the same gene locus cannot yet be answered.

Macro-orchidism

Nearly all post-pubertal affected males have large testes with a mean volume ranging from 15–127 ml (normal range 18–25 ml, depending on race) (Opitz & Sutherland, 1984). However, clinical diagnostic problems often arise in the pre-pubertal males. Thake *et al.* (1985) studying boys with severe mental retardation found 6 out of 10 boys with fragile X syndrome (aged 10–16 years) had testicular volumes over the 90th centile, whilst their 3 fragile X boys under 10 years had volumes of 4, 6 and 15 ml, all greater than 2 ml which is regarded as the upper limit of normal for this age group. However, another study (Largo & Schinzel, 1985) found 12/13 boys aged 2.6 to 12.5 years had testes volumes less than 2 ml. The situation is complicated by the fact that large testes are commoner in mentally retarded males as a whole. Howard-Peebles & Finley (1983) found that 8% of white males in a State mental institution had marked macro-orchidism (above 34 ml) but only 27% of these men were fragile site positive.

Physical features

The head circumference tends to be larger than normal, being greater than the 50th centile in about 75% of cases. In affected adult males Jacobs *et al.* (1983) found 4/9 and Meryash *et al.* (1984) 4/18 found head circumferences at the 97th centile, or above.

The face tends to be long with a long nose, slight mid-face hypoplasia and prominent mandible (Figure 2). The ears are large, with the mean length significantly increased (Meryash *et al.*, 1984) and are frequently anteverted and anteflexed. Submucous clefts, cleft uvula or palate may occur. These facial features are more common in adults with the Martin-Bell syndrome and may also occur in mentally retarded women.

Opitz *et al.* (1984) has drawn attention to what may be a generalized connective tissue dysplasia in the

Martin-Bell syndrome. There tends to be soft velvety lax skin, and hypoplasia of ear cartilage.

Neurological features

The degree of mental retardation is variable but the mean IQ is in the 35–50 range and speech development appears disproportionately delayed. Pueschel & Finelli (1985), studying 22 patients, found 82% had brisk deep tendon reflexes and 41% extensor plantar responses. Gaze eversion was observed in 77% and about half exhibited hyperactive behaviour; about half had some inco-ordination and a stooped posture and gait. Fryns *et al.* (1984) found 20/21 boys had hyperkinesia. Speech was delayed with 3 boys displaying no active verbal activity even after the age of 5 years. Echolalia and perseveration were found in 10/22. Largo & Schinzel (1985) found similar developmental behaviour disturbances in 13 boys from 3 families. Language, and imitative and symbolic play were particularly retarded. About 15–25% have a history of grand mal seizures, which can usually be controlled by anti-convulsants.

Criteria for cytogenetic investigation

Despite the emergence of a fairly characteristic phenotype in adult life, in paediatric practice it can still be difficult to decide in whom the fragile X should be sought. Thake *et al.* (1985) specifically addressed this problem using an appropriately ascertained sample of mentally retarded boys in a defined geographical area (Bundey *et al.*, 1985). In their study any boy with severe 'idiopathic' mental retardation, regardless of his clinical features, was associated with a 1 in 9 chance of identifying a fragile site at Xq27-8. If boys with a head circumference over the 50th centile, testicular volume over the 50th centile (for boys over 10 years) and an IQ between 35 and 70, were selected then the chance of finding a fragile X was 1 in 3.6. If an affected male had a similarly affected maternal male relative (other than his brother) the chance of finding fragile X was 1 in 2.6. The yield was only slightly less if one took all those with a similarly affected brother. Some cytogenetic laboratories have changed to folate depleted medium for all routine lymphocyte cultures, which at least allows a retrospective search for fragile sites if requested.

The fragile site at Xq27-28

A fragile site is a region that has failed to condense normally at the onset of mitosis, so the metaphase chromosome exhibits a constriction, in the present case at Xq27-8. High resolution banding studies have located the fragile site to band Xq27.3 (Brookwell & Turner, 1983). At least *in vitro* the linking strand is

Figure 2 Fragile X mental retardation. (a) Cousins from the original Martin-Bell pedigree showing typical facial features. (b) 3 year old boy who, apart from a relatively large head, has few dysmorphic features.

liable to break so that cells with a deletion of the tip of the long arm of the X can occur (Fitchett & Seabright, 1984) as well as those in which a single chromatid has broken away (Figure 3).

Typically 5–50% of the lymphocytes of an affected male show fragile sites. There is no explanation for why some cells of the same tissue do not express fragile sites. A fragile site probably represents defective DNA synthesis during the DNA replication that precedes mitosis, and perhaps variation in the length of the cell cycle thereafter allows some cells to complete DNA replication before the chromosomes condense.

At least one hamster/human somatic cell hybrid line was shown to express the fragile site in the absence of human autosomes (Nussbaum *et al.*, 1983), but it is by no means clear what all the factors necessary for fragile site expression are. Although the percentage of lymphocytes expressing fragile sites in affected males varies widely both between and, to a lesser extent (Soudek *et al.*, 1984), within families, one individual maintains a fairly constant level of fragile sites. Sutherland (1977) first showed that the expression of the fragile X is folate sensitive, being seen in lymphocytes that are cultured in media free of folic acid and thymidine. They can be induced by inhibitors of folate metabolism such as methotrexate and inhibitors of thymidylate synthetase such as FUdR and FCdR (Glover, 1983). Fragile sites can be induced

with difficulty in fibroblasts and amniocytes, usually using methotrexate or FUdR, but unfortunately these methods are too unreliable for prenatal diagnosis. Preliminary data (Tommerup *et al.*, 1985) suggest that it is possible to use trophoblast cell cultures obtained by chorionic villus sampling for prenatal diagnosis, and this is likely to replace fetal blood sampling as the prenatal diagnostic test of choice, although fetal lymphocyte analysis will always represent a valuable backup. It is not known what the nature of the fragile site is at the DNA level. Sutherland (1985) has recently observed that *excess* of thymidine, but not its analogue BrdU, will induce the folate sensitive fragile sites and concludes that the critical factor involved in their expression is the dCTP/dTTP ratio during DNA synthesis. He goes on to hypothesize that if either dTTP or dCTP was limited, single strand gaps would arise during DNA replication if the DNA sequence involved was an alternating polypurine/polypyrimidine structure such as (AGAGAG) n_1 -(TCTCTC) n_2 .

Cytogenetic/clinical correlations

Although it is natural to look for clinical correlations, there is no logical reason to suppose that there should be any relationship between the percentage of lymphocytes with fragile sites in an *in vitro* test and a

Figure 3 Two G-banded fragile X chromosomes viewed by scanning electron microscopy, showing the gap towards the distal ends of the long arms. The diagrammatic representation shows the bands viewed by light microscopy (chromatid i) and the bands viewed in prometaphase banding (chromatid ii).

clinical feature such as brain development. Clearly the presence or absence of any fragile sites is likely to be biologically meaningful, but other correlations, with age for example (Sherman *et al.*, 1984), may turn out to be spurious, being generated by ascertainment bias and the pooling of results from individuals in whom the genetic mutations are at different stages of evolution (Turner & Jacobs, 1983; Pembrey *et al.*, 1985).

Males Because many cytogenetics laboratories are now routinely using folate depleted medium, relatively large numbers of males have been screened and yet there are very few reports of a significant percentage of fragile sites being found in someone who is intellectually and psychologically normal. The family reported by Daker *et al.* (1981) was apparently entirely normal. The male described by Webb *et al.* (1981) suffered abnormal behaviour ascribed to shell shock, and other examples of boys with normal IQs were ascertained because they had autism. It is important to emphasize, however, that fragile X syndrome families may contain phenotypically normal males who have transmitted the Martin-Bell syndrome to grandchildren, but these normal transmitting males do not appear to have fragile sites (Froster-Iskenius *et al.*, 1984).

Females It has been estimated that, overall, about 30% of female heterozygotes show intellectual impairment (Turner *et al.*, 1980), although the degree of mental retardation is on average less than in hemizygous males. However, the occasional female can have severe mental retardation even more marked than an affected brother. Nearly all mentally retarded females in proven fragile X syndrome families show fragile sites (Turner & Jacobs, 1983) with the percentage of lymphocytes exhibiting fragile sites being in the 5–50% range, and on average not much less than in affected brothers. Within female sibships that include a mentally retarded female, there is no overall correlation of the percentage of fragile sites with IQs, although an inverse correlation does emerge when one relates the percentage of fragile sites on the *active X* chromosome to IQ (Paul *et al.*, 1984). The absence of the fragile site can either mean the X chromosome does not carry the definitive Martin-Bell mutation or that metabolic conditions in the cell do not allow expression of the fragile site. Overall, only about 50% of obligate carriers show fragile sites and this makes genetic counselling difficult.

Inheritance and genetic mechanisms

Until recently the Martin-Bell syndrome was regarded as a regular X-linked condition with manifestations in a proportion of female heterozygotes. Indeed, the complex segregation analysis used by Sherman and

colleagues (Sherman *et al.*, 1984, 1985) assumes classical X-linked inheritance. However, the original Martin-Bell pedigree showed transmission through two males 'reported to be normal' and by the end of 1983 there were eight pedigrees reported showing a total of 15 phenotypically normal transmitting males, plus another inferred from linkage data (Pembrey *et al.*, 1985), a most unlikely situation for a regular X-linked condition where, overall, one third of female heterozygotes are intellectually impaired (Turner *et al.*, 1980). Simple inspection of these pedigrees (Pembrey *et al.*, 1985) revealed that the 47 daughters of these normal transmitting males represented a special class of heterozygotes for they were not mentally retarded and had either no fragile sites or very few indeed. Furthermore, when these authors (Pembrey *et al.*, 1985) selected pedigrees from the literature that *might* represent transmission from a normal male (because they had at least two daughters who were obligate heterozygotes on pedigree grounds and no affected sons), they found a similar situation. These 16 normal men had 67 daughters and only one was reported as dull. It therefore has to be concluded that there are many more normal transmitting males than meets the eye, and pooling all pedigrees for the purpose of obtaining figures for genetic counselling is a waste of time, and may indeed give erroneous results. One would also expect segregation analysis of pooled data based on regular X-linked inheritance to give bizarre results, which is the case. Sherman and colleagues (1984, 1985) find a 20% deficit of affected males, that new mutations seem entirely confined to sperm, and that the mutation rate in sperm must be of the order of 7.2×10^{-4} , by far the highest rate recorded in mammals.

Analysis of pedigrees containing normal transmitting males suggests that what these men have inherited and transmit to their daughters is a premutation – namely, a change in the DNA that causes no harm *per se*, but predisposes to the definitive Martin-Bell mutation. Furthermore, the fact that the daughters of these normal transmitting males do not have mental retardation but their grandsons and granddaughters often do, indicates that the definitive mutation is only generated *in ova*. This in turn suggests that interaction between both X chromosomes is required to generate the definitive mutation, a recombination event being one, and perhaps the most likely, candidate (Pembrey *et al.*, 1985). Clearly the interaction of the X chromosome varies between females, because the mothers of normal transmitting males rarely have affected children, whilst the daughters of such males often do. This means that if this variability is inherent in the X chromosomes themselves, there must be a minimum of four types of X chromosome; premutation, an X that interacts with the premutation (probably the normal X), one that cannot interact with the

premutation, and the definitive Martin-Bell mutation. It is by no means proven that recombination events are responsible for generating these different X chromosomes, but models can be constructed that are potentially testable by the use of linked DNA probes.

Linkage studies

Physical mapping of genes and specific DNA sequences with respect to the fragile site at Xq27.3 have exploited the fact that the presence of the fragile site actually enhances the resolution of *in situ* hybridization. The gene for G6PD (Szabo *et al.*, 1984) and the polymorphic DNA probe, St 14 (Mattei *et al.*, 1985), have been cytologically mapped to Xq28, distal to the fragile site, and since the factor VIII gene and the probe, DX13, are very closely linked to St14 (Gitschier *et al.*, 1985), this places these at Xq28. The factor IX gene and probe 52A map to Xq27 centromeric to the fragile site (Mattei *et al.*, 1985).

With the exception of G6PD (which reveals a protein polymorphism), all the above probes reveal common restriction fragment length polymorphisms, and are therefore useful DNA markers for linkage studies within families.

There were early claims that linkage to factor IX was close enough to be clinically useful (Camerino *et al.*, 1983), but these have not been substantiated (Davies *et al.*, 1985). More closely linked probes will be needed before direct DNA analysis can supplement cytogenetic studies for carrier detection and prenatal diagnosis.

Rare deletions or translocations involving the X chromosome

Paediatricians may, once or twice in a lifetime, come across extraordinary experiments of nature, where a single child appropriately investigated will provide insight into basic mechanisms. There are at least two situations involving the X chromosome.

Rarely children are seen with more than one X-linked disease and this should always raise the suspicion of a deletion either detectable by cytogenetic analysis or submicroscopic and therefore only detectable with X-specific DNA probes.

In 1980, Guggenheim *et al.* reported glycerol kinase

deficiency in two boys with adrenal hypoplasia, mental retardation and neuromuscular problems, which on muscle biopsy were histologically compatible with Duchenne muscular dystrophy. Renier and colleagues (1983) have described 3 male sibs with the same spectrum of disease. One link between these apparently diverse phenotypes is the X-linked inheritance. Pseudohypertriglyceridaemia (Rose & Haines, 1978), congenital adrenal hypoplasia (Hay *et al.*, 1981; Hensleigh *et al.*, 1978) and Duchenne muscular dystrophy are all inherited as X-linked recessives. Bartley *et al.* (1982) noted the concordance of X-linked glycerol kinase deficiency with X-linked congenital adrenal hypoplasia in their studies of two families, but were unable to detect any abnormalities on chromosome studies. More recently, Hammond *et al.* (1985) reported a male infant with glycerol kinase deficiency, adrenal hypoplasia and another X-linked condition, ornithine carbamoyl transferase deficiency (OCT) (ornithine transcarbamylase deficiency, OTC, EC 2.1.3.3) who had a deletion on the short arm of the X chromosome. Dunger *et al.* (in preparation) have reported two unrelated boys who have glycerol kinase deficiency, adrenal hypoplasia, mental retardation and muscular dystrophy. In one of these boys they were able to detect a deletion on the X chromosome by direct analysis using the probe 754 even though a deletion was not visible by ordinary cytogenetic analysis. Such cases are likely to prove very useful in pinpointing the Duchenne muscular dystrophy locus and allowing this important gene to be cloned.

The first hint that the Duchenne muscular dystrophy locus was on the short arm of the X chromosome at p21 came from the rare cases of girls with the full clinical features of Duchenne muscular dystrophy. Several such cases had an X:autosome translocation (Greenstein *et al.*, 1977; Zatz *et al.*, 1981) and although the autosome involved varied, the breakpoint on the X was always at Xp21. It seems that the normal X chromosome becomes inactive in all cells, and the translocated X chromosome has the normal gene at the Duchenne muscular dystrophy locus disrupted by the translocation.

Localization of the Hunter gene locus to the long arm of the X chromosome came from a girl with Hunter's disease who carried an X:5 translocation (Mossman *et al.*, 1983).

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