Review article

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Molecular genetics of the human X chromosome

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SUMMARY The human X chromosome will soon be mapped at 10 cM intervals. This will permit the localisation of any X linked disorder provided that informative families are available for linkage analysis. The location of RFLPs currently in use for clinical diagnosis is summarised. The next decade should witness the elucidation of the molecular basis of some of the more common defects, such as the muscular dystrophies and X linked mental retardation.

Genes and phenotypes can be mapped to the X chromosome by their characteristic sex linked mode of inheritance in families. This is not possible for autosomal sequences and consequently the X chromosome is the most extensively mapped of all human chromosomes. There are at least 100 disease loci localised to the X chromosome and at least 100 DNA markers. Until a few years ago there were two main linkage groups, one localised at Xpter and the other at Xqter. With the application of restriction fragment length polymorphisms (RFLPs) as genetic markers, a map spanning the entire X chromosome is now almost complete. The markers are sufficiently well distributed along the chromosome to enable the mapping of almost any sex linked disease (fig 1).

Conventional genetic markers

Until the advent of DNA recombinant technology, many disease loci had been assigned to the human X chromosome, but few had been shown to belong to established linkage groups. This was because the main linkage groups were localised at the telomeres and there was a paucity of intervening markers.

The Xg blood group was first described in 1962 and localised to the region Xp22.3–Xpter by linkage to X linked ichthyosis (steroid sulphatase [STS] deficiency).^{1–3} Deletion mapping assigns Xg distally to the STS locus and both of these escape X inactivation. Other loci in this linkage group include the Xk blood group (Xk locus), chronic granuloma-

FIG 1 Map of some clinically useful DNA probes on the human X chromosome.

tous disease (*CGD*), ocular albinism (*OA*), and X linked retinoschisis (*RS*).^{4 5}

The other important linkage group is localised in the telomeric region of the long arm and consists of the loci coding for colour blindness, haemophilia A, glucose 6 phosphate dehydrogenase (*G6PD*), and adrenoleucodystrophy.^{6 7} Goss and Harris,⁸ using radiation induced gene segregation, showed that the hypoxanthine guanine phosphoribosyltransferase (*HPRT*) locus is localised proximally but close physically to the *G6PD* locus. However, Francke *et al*⁹ showed that these loci are not closely linked genetically. This was the first hint of the increase of recombination towards the end of the X chromosome long arm.

Gene specific probes and clinical diagnosis

Several gene specific probes have been isolated for the direct analysis of X linked diseases. These include the genes coding for *PGK* (phosphoglycerate kinase),^{10 11} *OTC* (ornithine transcarbamylase),¹² *HPRT*,^{13 14} *G6PD* (glucose 6 phosphate dehydrogenase),¹⁵ *F9* (factor IX),^{16 17} and, more recently, *F8* (factor VIII).¹⁸⁻²¹

Genetic defects at many of these loci have been studied in detail. Only a very small proportion of them are caused by deletion of all or part of the gene sequence. Yang *et al*²² showed that different mutations at the *HPRT* locus are responsible for Lesch-Nyhan syndrome. In general, many defects cannot be detected by alterations in the restriction enzyme sites at or around the affected locus. Thus, antenatal diagnosis of genetic disorders is often carried out using linked restriction fragment length polymorphisms revealed by the relevant gene specific probe.²³ An example of this approach is the diagnosis of factor IX deficiency (Christmas disease) by the segregation analysis of a TaqI RFLP detected by the factor IX gene probe²⁴ (see references 25 and 26 for detailed discussion of this strategy). The major limitation of this approach is that insufficient RFLPs are yet characterised to ensure that every affected family is informative. In addition, in some cases, family studies are not informative because of the absence of normal or affected male offspring who are needed to determine phase, since the RFLPs are not in linkage disequilibrium with the disease.

Isolation of random X chromosome DNA probes

The first methods used for the isolation of X chromosome sequences depended upon a method developed by Kunkel *et al*^{27 28} to obtain Y chromosome specific DNA. Schmeckpeper *et al*²⁹ isolated X chromosome sequences by reassociating radio-

labelled DNA from hybrid cells containing the human X chromosome with an excess of unlabelled DNA from cells devoid of the human X chromosome. Gusella *et al*³⁰ have demonstrated that it is now possible to identify human chromosome sequences in a somatic cell hybrid by cloning the total DNA and screening the clones with a human specific repeat such as Alu.

A more direct method of obtaining X chromosome specific sequences is to sort the chromosomes using a flow activated cell sorter.^{31 32} The chromosomes are stained with a fluorescent dye which is excited by a laser beam. The X chromosomes are then collected according to their characteristic fluorescence, stripped of their protein coat, cut into fragments using a restriction enzyme, and cloned into a phage vector. This library of DNA sequences has proved to be a very useful basic resource for the isolation of random DNA segments for the genetic mapping of the X chromosome.³²⁻³⁴

Physical mapping of the human X chromosome

Three main methods have been used. The first depends upon the detection of the number of copies of a region of the X chromosome present in DNA derived from cell lines which contain X chromosome deletions.^{33 35} If the deletion is present in a male, then the DNA sequences can be assigned to this region by lack of hybridisation to the DNA in a Southern blot. Whenever the deletion is present in a female, then the sequence is assigned by dosage. The hybridisation signal will be half of the normal (46,XX) signal if the signal lies in the deleted region. The second method depends upon having deleted or translocated X chromosomes present in somatic cell hybrids. This approach was used by Wieacker *et al*³⁶ to localise random DNA probes regionally along the X chromosome length. Finally, the more direct method of hybridising the DNA sequences in situ to metaphase chromosomes has also been used.³⁷⁻³⁹ Hartley *et al*⁴⁰ used this technique to demonstrate the relationship between the physical and genetic map on the human X chromosome.

Genetic mapping using RFLPs

More than 20 RFLPs have now been characterised on the human X chromosome and the Eighth International Gene Mapping Conference in Helsinki this year promises to be one in which more RFLPs will be reported and their order along the chromosome established. Drayna *et al*,⁴¹ using eight RFLPs and conventional markers, constructed a map of the human X chromosome where the total genetic length was estimated to be about 200 cM. They

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observed an increase in the frequency of recombination towards the telomeres similar to that observed previously for autosomes.^{42 43} In particular *factor IX*, which lies proximally to Xq27, and *DX13*, a marker very closely linked to *haemophilia A*, were found to be only loosely linked in spite of the relatively short physical distance between them.⁴⁴ On the short arm, *RC8*, localised at Xp22.3, is not closely linked to *Xg*⁴⁵ and only shows loose linkage with the steroid sulphatase locus (*STS*) and the retinosischisis locus (*RS*).^{46 47}

Mapping of Duchenne and Becker muscular dystrophies

Duchenne muscular dystrophy (*DMD*) is one of the most common X linked recessive disorders and affects approximately one boy in 3000.⁴⁸ The underlying biochemical defect still remains elusive and before 1982 there was no linkage information except negative data with *Xg*. However, one clue to the localisation of the mutation was provided by the existence of affected females with balanced X; autosome translocations with breakpoints on the short arm of the X chromosome at Xp21.⁴⁹ It was proposed that the X chromosome breakpoint at Xp21 affects the expression of genes in this region, either by disrupting them directly, or by position effects.

The first RFLP found to be linked to the *DMD* locus was *RC8*, a DNA sequence localised at Xp22.3 proximal to *Xg*.^{34 40} Studies of the segregation of this locus in British *DMD* families demonstrated linkage at approximately 17 cM.³⁴ This work was extended using a second probe, *L1-28*, physically localised at Xp11.4 on the opposite side of the locus from *RC8*. Linkage analysis of *L1-28* showed it to be also approximately 17 cM away from *DMD*.⁵⁰ These data confirm the assignment of the mutation to the Xp21 band.

At this time Becker muscular dystrophy (*BMD*) was thought to be located on the long arm because of its apparent linkage to colour blindness in one pedigree. However, experiments by Kingston *et al*.^{51 52} demonstrated that *BMD* segregates in the same manner as *DMD* with *RC8* and *L1-28*. Both of these loci must therefore lie within Xp21 and may be allelic. Analysis of these diseases with more closely linked markers confirms this result.

There are now at least 11 RFLPs bridging *DMD* and *BMD* for use in carrier status determination (fig 2).^{53 54} Even when there is minimum information with RFLPs and from the creatine kinase level in serum, the two sets of data can be combined to improve the information available.^{55 56} In some instances, the mother is informative for enough

bridging markers for antenatal diagnosis to be possible.⁵⁷ However, none of these probes is tightly linked to the mutation^{58 59} even though one of them (754) lies within an X chromosome deletion at Xp21.2 in a male *DMD* patient.³⁵ Furthermore, several families have been studied where double crossovers occur in the interval 99-6 and *L1-28* and *D2* and *L1-28*⁵⁷ (K E Davies, unpublished results). These data may indicate a high frequency of recombination in this region of the human X chromosome in families segregating for the *DMD* mutation. Alternatively, the results could be explained if rearrangements, such as inversions, were occurring. One of the female patients with muscular dystrophy with an X;1 translocation does have a

FIG 2 Localisation of RFLPs useful for linkage studies in Duchenne and Becker muscular dystrophy families.

rearrangement of the short arm of the X chromosome that has been interpreted as a possible inversion.⁶⁰

However informative RFLPs are in families, they still do not solve the problem of new mutations that present at the clinic, nor do they give an unequivocal diagnosis.⁶¹ For this reason, there is a great need for a more direct analysis of the mutation itself. One strategy for the identification of the mutant gene is to isolate the breakpoint in the female translocations. This approach is being used with the X;21 translocation by Worton and co-workers.⁶² In this patient, the ribosomal cistrons on chromosome 21 are translocated to the X chromosome. Thus, the breakpoint fragment on the derivative X chromosome should hybridise to X chromosome material as well as rDNA. The cloning of such a fragment would be of great interest in view of the cytogenetic studies by Boyd and Buckle,⁶³ which suggest that the breakpoints in these female patients might not be identical.

Other strategies for isolating the DMD mutation include the cross screening of cDNA libraries with genomic X chromosome libraries and the comparison of genomic DNA from patients with deletions and normal subjects.⁶⁴ There are now DNA markers within 2000 kb of the *DMD* and *BMD* loci. Thus, the first mutation should be identified very shortly if it is an easily recognisable variation in the normal DNA sequence. It will then be possible to explore the degree of heterogeneity in the disease at the molecular level and to begin to understand the biochemical basis for its clinical course.

Fragile X mental retardation

The most common form of X linked mental retardation is associated with a fragile site at Xq27.⁶⁵ This fragile site is only seen in vitro after culturing the lymphocytes of persons with the mutation in folate deficient media. However, even under these conditions, not all affected subjects show the fragile site and its actual relationship with X linked mental retardation is unclear. Fewer than 1% of males express the fragile X and have a normal IQ.⁶⁵⁻⁶⁸ Moreover, there are reports where mentally normal males have transmitted the fragile X to their daughters. Thus, this disorder does not display normal X linked inheritance. The existence of a premutation in the normal males carrying the defect has been proposed to explain these observations.⁶⁹⁻⁷⁰

Linkage studies between *HPRT* and the fragile site suggest that these loci are not closely linked.⁷¹ A random DNA probe, 52A, which lies distal to *HPRT* but proximal to the fragile site⁷² only shows

loose linkage with the fragile site.⁴⁴ *Factor IX*, which also lies proximal to the fragile site but distal to 52A, was first reported to be very closely linked to the locus.⁷³ However, several other reports suggest that the families in which close linkage was observed are exceptional.^{44 74 75} In other kindreds only loose linkage was observed between 52A, *factor IX*, and the *fragile X*.⁴⁴ Studies in families also segregating for *G6PD* have led to the proposal that the fragile site is a hot spot for recombination and that other markers on either side of it segregate proportionally to physical distance.⁷⁶⁻⁷⁸ Other studies support the concept of a hot spot of recombination, but an increase in recombination between markers adjacent to the locus has also been observed.⁴⁴ More families are required to clarify the linkage relationships. The conclusion from the studies so far is that there are no clinically useful closely linked markers for this disorder.

Other X linked disorders

RFLPs have been used in the analysis of several X chromosome disorders. Retinitis pigmentosa is closely linked to *L1-28*. Bhattacharya *et al*⁷⁹ studied four families segregating for this disease and observed a maximum lod score of 7.89 at 3 cM. However, a patient with a deletion at Xp21-2 suffers from DMD and retinitis pigmentosa.³⁵ This is consistent with McKusick's suggestion of multiple loci for X linked RP.⁸⁰ Very recently, Norrie's disease was localised in this region by linkage analysis of *L1-28* in affected families.⁸¹ Markers closely linked to the *haemophilia A* locus have been reported and are already being used for antenatal diagnosis and carrier detection.^{82 83} The factor VIII gene itself is also polymorphic and will become the probe of choice in the future. The use of closely linked probes, such as *DX13*, where no recombinants have been found in at least 50 meioses, demonstrate the potential use of RFLPs even when a gene specific probe becomes available. *DX13* will continue to be of value unless the gene specific probe can give information in every case. A second probe (*St14*) is also closely linked to *haemophilia A* and is very polymorphic, showing at least 10 alleles in a Southern blot after digestion of DNA with *TaqI*.⁸⁴

X/Y homologous sequences

The terminal portion of the short arm of the human X chromosome undergoes synapsis at male meiosis and it has been proposed that the X and Y chromosomes possess homologous sequences in this region.⁸⁵⁻⁸⁷ Indeed, there is evidence for the existence of a regulatory 'Yg' locus and for the homology

of *MIC2X* and *MIC2Y*.^{88 89} Cloning of other DNA sequences from the X and Y chromosomes has demonstrated that X/Y homology is not confined to the meiotic pairing region.^{90 91} This will be discussed more extensively by Goodfellow and colleagues in the next review in this series.

Gene therapy for X linked disorders

Gene therapy, the replacement of a non-functional gene with a functional one, is a technological advance for the future. However, it is worth mentioning briefly here because it is already being attempted in somatic cells for Lesch-Nyhan syndrome (HPRT deficiency). This is a good candidate for initial experiments because it is a disorder where there is an enzyme missing and it is possible that even low levels of HPRT would improve the patient's condition. In addition, there is no evidence that an excess of HPRT would be harmful. Thus, the new gene could be introduced and, even if its expression was not controlled rigorously, the patient might still benefit. One method of introducing functional HPRT genes into bone marrow cells is to use retroviral vectors.⁹²⁻⁹⁴ The retroviral vectors containing the HPRT genes integrate into the bone marrow genomic DNA at random. Before this gene therapy approach becomes feasible it will be necessary to control this integration in some way so that the new DNA does not insert into a critical region of a chromosome.

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