Genetic mechanisms and mental retardation

ABSTRACT—The past five years have witnessed rapid and apparently relentless progress in the delineation of the genetic basis of disorders associated with mental retardation. Each gene discovery has a new story to tell but inevitably generates further questions. For the clinical geneticist and, perhaps more importantly, for patients and their families, many of these recent discoveries have yielded information which has immediate implications for diagnostic testing, family and population screening and prenatal testing. Many of the ethical issues consequent upon the rapid progress are only now being addressed. This article highlights a number of disorders whose molecular genetic basis has recently been further characterised. Brain development and maintenance of neurological networks provide the unifying theme; the genetic defects are disparate and each of their mechanisms appears to be novel.

Microscopic deletions

Aneuploidy for whole chromosomes or large chromosomal regions led to the initial characterisation of major causes of mental retardation. The recognition of such chromosomal disorders remains imperative for appropriate counselling and antenatal diagnosis. More recently, technical developments, including fluorescent in situ hybridisation (FISH) and gene mapping, achieve resolution far greater than the 1–2 megabase resolution obtainable through the best chromosomal banding of high quality prometaphase spreads. Consequently, more syndromes with consistent involvement of a small chromosomal region have been recognised [1]: Rubinstein-Taybi (chromosome 16p13.3, peculiar facies; broad thumbs and mental retardation); WAGR syndrome (11p, Wilms tumour, aniridia, genitourinary malformations and retardation); Williams syndrome (7q35, supravalvular aortic stenosis, characteristic facies and retardation) are all typical examples. Delineation of the genes involved in these submicroscopic deletions is under way.

Similar deletions have also been identified in the Prader-Willi and Angelman syndromes. These two disorders provide clear evidence for an important *parent of origin* effect (parental imprinting) in determining disease phenotype.

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Parental modification—genetic imprinting and brain development

Angelman syndrome and Prader-Willi syndrome both cause mental retardation with distinctive and quite different clinical phenotypes. In at least 50% of children with Angelman syndrome, deletion of 15q11–13 may be detected through either cytogenetic or molecular analysis. The same cytogenetic deletion is found in 60% of children with the Prader-Willi syndrome with the clinical features of short stature, obesity, hypogonadism, hypotonia and mental retardation. Whilst clinically distinct, the only demonstrable genetic difference is that the deletion in Prader-Willi arises on the *paternal* chromosome 15 and that in Angelman it arises on the *maternal* chromosome 15 [2].

Both disorders have now been shown to arise from deletions of distinct loci, but may also arise through an error in the segregation of chromosome pairs, leading to uniparental disomy, when the offspring has two chromosomes from one parent and none from the other [3].

Evidence for genomic imprinting, in which the same gene has different effects which depend upon the gene's parental origin, is extrapolated from research in mice. The observations of 15q11–13 deletion syndromes imply that normal brain development requires appropriate parental genetic complements from this region, perhaps in a phased manner. Characterisation of the specific loci involved will allow this hypothesis to be formally examined.

Fragile X syndrome

This is a serious and common cause of mental retardation, second only to the chromosomal disorder Down's syndrome. Prevalence estimations of 0.3–1/1,000 in males and 0.2–0.6/1,000 in females are widely accepted [4].

The term fragile X is derived from the unusual appearance of the tip of the long arm of the X chromosome in a variable proportion of the cells inspected from an affected individual. The fragile site consists of a constriction at Xq27 with partial detachment of the distal portion. However, special conditions for cell culture preparation, in folate and thymidine depleted medium, are required to demonstrate this classical appearance. Even then, and of importance for diagnostic or population screening, the fragile X chromosome is not seen in every cell. In affected males some 4–50% of cells may show the fragile site, in female

gene carriers of the disorder the proportion is less and in 50% of these females the fragile X cannot be detected at all.

Clinically, the retardation is non-specific and varies from mild to severe in males and mild to moderate in females [5]. The absence of characteristic dysmorphic features makes it difficult to delineate suggestive features for the clinician. Affected children may be slightly taller than usual, perhaps with loose joints. Speech is typically cluttered and repetitive. In post-pubertal males, the testes are usually significantly enlarged and the face is long with a large, squared-off mandible and large ears. Microcephaly, dysmorphic facial features or abnormal neurological features make the diagnosis unlikely and would not typically lead to a request for either cytogenetic or DNA based fragile X analysis [5].

An early report of the fragile X syndrome, then also known as the Martin Bell syndrome, came in 1943. It referred to a large pedigree with several affected members, compatible with a 'semi-dominant' X-linked inheritance. However, the family (see Fig 1) also revealed completely normal men yet who passed on the X chromosome with the fragile X disease gene. Such men, referred to as normal transmitting males, pass the gene on to their normal daughters who may then go on to have affected sons. This paradoxical form of X-linked transmission was analysed in detail by Sherman *et al* [6], who considered that the mutation in the fragile X gene must exist in two distinct forms, the first a 'premutation' and the second the 'full mutation' leading to the disorder.

Dynamic mutation

Molecular defect leading to the fragile X syndrome

The defect in this disorder has important implications for understanding brain function. It is now known to be the result of a novel genetic mechanism with parallels in a number of common neurological disorders. Variable expansion in a specific repeat of the DNA sequence (CGG) occurs in a gene termed *fmr-1*, whose function is still unclear. The size of the expansion varies between different cells (Table 1) and typically progresses from one generation to the next. Hence, subjects from the same family who carry the mutation have it in different forms. Normal transmitting males have the minimal expansion (premutation) but after transmission and processing by their clinically normal daughters the DNA triple repeat may undergo significant expansion and generate a phenotype of highly variable severity. In practical terms it is now possible to indicate the degree of risk to individuals and offer specific molecular tests for carrier detection, diagnostic

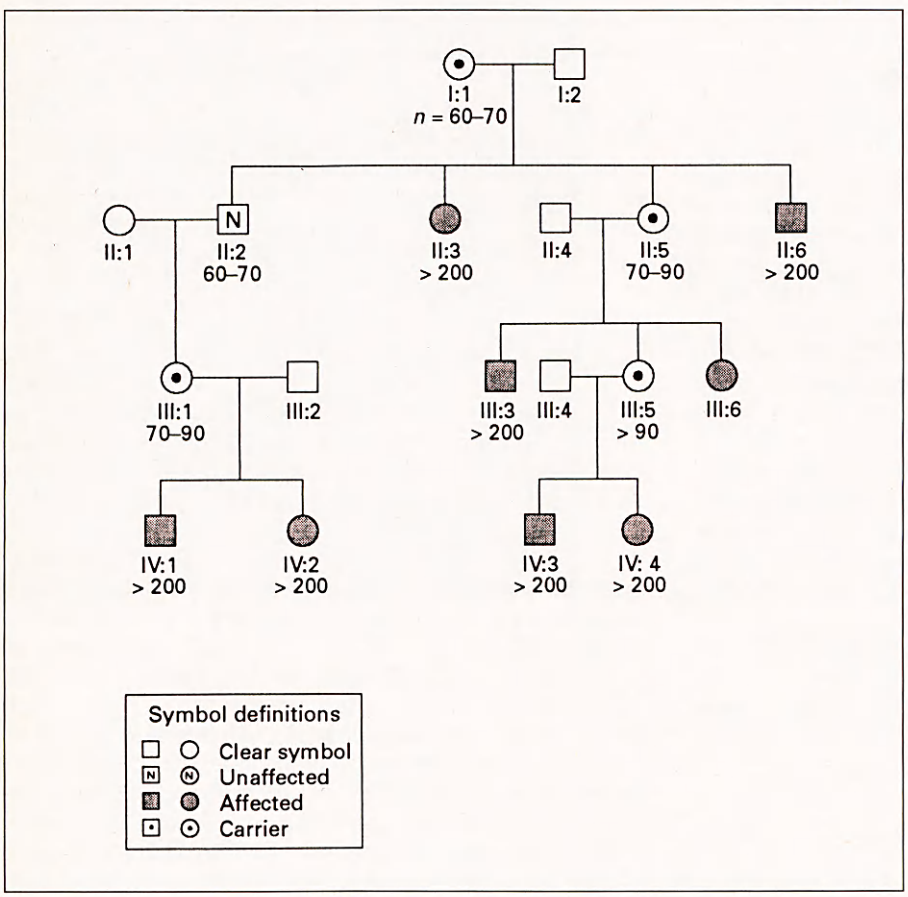

Fig 1. *Fragile X family with size of FMR1 mutation repeat assayed in lymphocytes. Normal transmitting males (N) harbour the permutation but appear phenotypically unaffected (see text).*

Table 1. Genetic mechanisms in mental retardation

Chromosomal	Chromosome number	eg Trisomy 21	Trisomy 21
	Deletion	eg 5p-	Cri du chat
	Microdeletion	eg 16p13 17p	Rubenstein Taybi Miller-Dieker
Parent of origin	Microdeletion	eg 15q	Angelman Prader-Willi
Dynamic DNA mutation (unstable DNA)		eg Xq27 16p 19	Fragile X Huntington's disease Myotonic dystrophy
Tumour suppressors and neuronal tissue		eg 17 16	Neurofibromatosis Tuberous sclerosis

assessment, prenatal testing and identification of minimal mutation expansion in chromosomally normal individuals from at risk families. It is therefore feasible to use DNA based tests for screening mentally retarded individuals or even for carrier detection in the general population [7].

In comparison to most other single gene defects, the unstable DNA element affords both advantages and disadvantages for diagnostic purposes. In cystic fibrosis, the most prevalent autosomal recessive disorder in the UK, the genetic defects are variable and numerous; the F508 deletion predominates but over 200 other mutations have been described. Technically, diagnostic tests have yet to be developed. In contrast, the dynamic mutations in fragile X are homogeneous; only a few examples of gene deletion and point mutation have been reported. It is relatively easy to assay the dynamic mutation. The principal disadvantage is the complex relationship between genotype and phenotype reflecting the widespread somatic variation in the size of the repeated DNA sequence.

At least seven neurological disorders due to dynamic mutations of repeated elements have been described [8]: spinobulbar muscular atrophy, spinocerebellar ataxia, dentatorubral–pallidolysium atrophy and FRAX E each being less common than the three detailed in Table 2.

Huntington's disease

Huntington's disease (HD) is among the most serious and frequent neurogenetic disorders of adult life. The disorder is characterised by progressive motor deterioration with involuntary movements, mental involvement with dementia, and widespread neurodegeneration [9]. Onset may be at any stage but is typically in mid to late life, with consequent severe disability and frequently profound psychological effects for family members.

The HD gene has recently been isolated, and named *it15* [10]. The gene comprises 67 exons extending over a large (100kb) region of genomic DNA. The disease mutation is an expansion of an AGC repeat sequence within the protein coding sequence. The molecular genetic details of this dynamic mutation are now emerging. For example, the juvenile onset form of HD is paternally determined. The

Table 2. Dynamic DNA mutations in disorders associated with neurological dysfunction

	Fragile X	**Huntington's disease**	**Myotonic dystrophy**
Inheritance	X-linked dominant incomplete penetrance	Autosomal dominant	Autosomal dominant
Triplet repeat	(CGG)	(AGC)	(AGC)
Size normal alleles	10–50	9–34	5–35
Disease alleles	52–200 (premutation) 200–2000 (full mutation)	30–100	50–80 (premutation) 80–2000 (full mutation)
Expressed protein	RNA binding protein—widely expressed in tissues	Unknown function widely expressed	Protein kinase
Transmission of 'severe'	Maternal (full mutation)	Paternal (early onset)	Maternal (congenital)

p(AGC)n repeat in HD sperm is particularly unstable and may be the reason why there are greater differences in copy number between fathers and their children than between mothers and their offspring [11]. Although a large expansion is associated with the juvenile form, little relationship could be derived between age of onset and copy number below a repeat of 50, and determinants of genotype/phenotype correlation remain to be explored. The role of *huntingtin,* the gene product of the *it15,* in brain development will similarly emerge over the next few years and one may hope for the development of effective treatment from such progress. For the present, it remains important to apply high standards when undertaking presymptomatic testing due to the ethical and counselling dilemmas created.

Myotonic dystrophy

Myotonic dystrophy (DM) is an autosomal dominant disorder with a prevalence of 0.13/1,000 live births [12]. It causes progressive weakness and wasting of skeletal muscle with myotonia. The gene for DM maps to chromosome 19q13.3. Positional cloning has helped to identify the DM gene and localised a dynamic mutation (AGC repeat) to the 3' untranslated region [13]. The repeat size typically increases from generation to generation, providing a molecular basis for the clinical phenomenon of *anticipation* of clinical severity [14]. However, decreases in the size of the repeat have also been noted, although in only four families has this led to a reduction to within the recognised normal range. At least for the DM protein kinase gene product, the expanded repeat mutation appears to result in undetectable levels of processed mRNA [15].

In summary, in the short period since the discovery of the fragile X mutation, a novel genetic element and mechanisms of mutation of considerable significance toward understanding both normal and disrupted brain development have been uncovered.

Tumour suppressor genes and the brain

Molecular studies have identified an important group of genes with a regulatory role in cell cycle control. As a group they may be referred to as tumour suppressor genes. The clue to the location of such genes has come from recognition of chromosomal changes seen in a number of cancers, with evidence that loss of genetic material leads to loss of tumour suppression. A group of disorders frequently referred to as phakomatoses has been further united through this common molecular mechanism. Each condition, neurofibromatosis type 1 (NF1) and type 2 (NF2) and most recently tuberous sclerosis (TSC), arises consequent upon mutation in tumour suppressor genes (Fig 2).

The two hit mutation concept

Mutation of a tumour suppressor gene is transmitted as an autosomal dominant trait in the germ line, but before a tumour can develop an additional somatic gene defect is necessary which makes both alleles nonfunctional (loss of allele heterozygosity). In sporadic examples of these tumours two independent somatic mutations are required, but individuals who have already inherited one gene defect need only sustain a

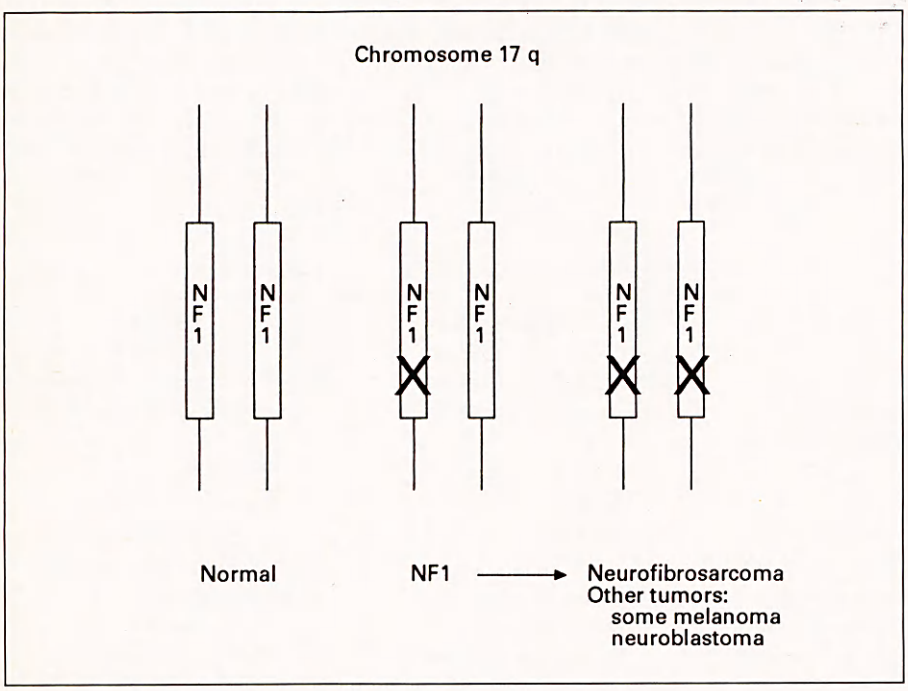

Fig 2. *Loss of allelic function of a tumour suppressor gene and its association with disease. NF1 = Neurofibromatosis Type 1.*

further single somatic event to initiate tumour formation. This readily explains why in the inherited forms tumours tend to be earlier and multiple.

Tuberous sclerosis

TSC is an autosomal dominant disorder characterised by the widespread development of growths, usually referred to as hamartomata, in many tissues including the brain, eyes, skin, kidneys, heart and lungs, giving rise to a highly variable clinical picture both in site and severity. In some, the classic skin lesions—multiple depigmented patches, adenoma sebaceum, shagreen patches and subungual fibromata—may be the only abnormalities, in others severe epilepsy or mental retardation may arise from cerebral tuber formation. The condition is likely to be underdiagnosed: its true prevalence may be as high as 1 in 5,800 [16].

Family linkage studies have established locus heterogeneity with disease-determining genes on chromosomes 9 and 16, either of them leading to indistinguishable clinical phenotypes. Refinement of the linkage maps has localised TSC 1 and TSC 2 to a small region of the telomere chromosomal bands—9q34.3 and 16p13.3. Recently, loss of heterozygosity has been identified in a proportion of hamartomata from TSC patients, providing strong support that a somatic mutation is required to produce the TSC phenotype—a notion consistent with the chromosome 16 gene acting as a tumour suppressor.

Deletions have now been observed which involve a putative TSC gene at 16p13.3 with a widely expressed transcript. The protein product has been termed *tuberin* [17]. DNA based diagnostic methods for assessment of relatives of TSC patients can be expected but it will be necessary to identify the TSC 1 gene also on chromosome 9 before the full mutational spectrum of TSC can be appreciated.

Gene-gene interaction and normal brain development—X-autosome regulation (ATR-X syndrome)

Detailed clinical and molecular genetic assessment of patients with alpha thalassaemia and mental retardation (ATR-X) has delineated another novel mechanism for mental retardation due to a genetic interaction, hitherto the subject only of speculation [18]. Clinical features in patients without detectable deletions of the α globulin genes on chromosome 16p include severe mental retardation, microcephaly, genital anomalies and seizures. All patients show mild features of HbH disease (HbH inclusions in red cells on staining with 1% brilliant cresyl blue) and all are chromosomal males. Linkage analysis in several extended families has identified an X chromosome locus capable of influencing the synthesis of α-globin chains coded for by the chromosome 16 genes [18]. The characterisation of this *trans* acting element will help unravel a further mechanism of mental retardation.

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