MONOGENIC CAUSES OF X-LINKED MENTAL RETARDATION

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Mutations in X-linked genes are likely to account for the observation that more males than females are affected by mental retardation. Causative mutations have recently been identified in both syndromic X-linked mental retardation (XLMR) and in the genetically heterogeneous 'nonspecific' forms of XLMR, for which cognitive impairment is the only defining clinical feature. Proteins that function in chromatin remodelling are affected in three important syndromic forms of XLMR. In nonspecific forms of the disorder, defects have been found in signal-transduction pathways that are believed to function during neuronal maturation. These findings provide important insights into the molecular and cellular defects that underlie mental retardation.

PHENYLKETONURIA
An inborn error of metabolism caused by lack of the enzyme that converts phenylalanine to tyrosine. It causes abnormally high phenylalanine levels and severe, progressive mental retardation if untreated, but can be prevented by neonatal screening and a low phenylalanine diet from an early age.

HOLOPROSENCEPHALY
A failure of the forebrain (prosencephalon) to divide into hemispheres or lobes, often accompanied by a deficit in midline facial development.

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Mental retardation (MR) is the most frequent cause of serious handicap in children and young adults. Defining features of MR include an overall intelligence quotient (IQ) of less than 70 (BOX 1), together with associated functional deficits in adaptive behaviour (such as daily living, social and communication skills), which manifest before 18 years of age (see REFS 1,2 for a review). Moderate to severe MR (IQ < 50) is estimated to affect 0.3–0.5% of the population, and the prevalence increases to 1–1.5% if mild MR (IQ = 50–70) is included, although these estimates vary widely between epidemiological studies[2,3].

The underlying causes of MR are extremely heterogeneous. There are several non-genetic factors that act prenatally or during early infancy to cause brain injury: these include infectious diseases (such as cytomegalovirus infection during pregnancy and postnatal meningitis), very premature birth, perinatal anoxia, and fetal alcohol syndrome, which is caused by excessive maternal alcohol consumption during pregnancy. Well-established genetic causes of MR include visible chromosomal anomalies and monogenic diseases. A search for MR in the Online Mendelian Inheritance in Man (OMIM) database identifies almost 1,000 entries that include MR as a clinical feature (which might be present in all or in only a subset of patients). However, 25–40% of cases with severe MR, and most mild MR cases, remain unexplained. Most of these mild cases probably involve combinations of multigenic and environmental factors.

Our knowledge of the monogenic causes of MR has increased markedly in recent years, notably through the use of positional-cloning strategies, which have allowed many disease genes associated with MR to be identified. Impaired cognitive functions are seen in metabolic diseases (such as the Smith–Lemli–Opitz syndrome, a defect in cholesterol metabolism, or in untreated PHENYLKETONURIA), as well as in developmental disorders (such as HOLOPROSENCEPHALY) or neuromuscular diseases (such as Duchenne muscular dystrophy); in the latter two conditions, however, MR is not a constant feature. Chromosome microdeletions are an important genetic cause of MR. They are involved in syndromes such as Prader–Willi and Angelman, as well as in Williams–Beuren syndrome (also known as Williams syndrome) which is associated with a more subtle cognitive impairment[4,5]. Recent studies indicate that chromosomal rearrangements that affect the telomeric regions of autosomes and that are not detectable by conventional cytogenetic analysis, might account for up to 7% of moderate to severe MR[6]. Some diseases for which a causative gene has been identified affect relatively large numbers of patients and families, such as the fragile X syndrome (which affects 1 in 4,000 males and 1 in 7,000 females). However, our knowledge of the monogenic causes of MR is still far from complete. Identifying the genes involved, especially for those cases in which MR is the only obvious symptom, is a key challenge for human

Box 1 | **Determining intelligence quotient**

Intelligence quotient (IQ) is measured using a range of tests (the best known are the Wechsler adult or child intelligence scale, and the Stanford Binet intelligence scale). These tests assess verbal and performance (non-verbal) IQ[87,88]. Other scales can be used to estimate adaptive behaviour. In principle, IQ tests are normalized so that the mean IQ in the population is 100, and the standard deviation is 15. Assuming a Gaussian distribution of IQ, about 2.3% of the population would be expected to have an IQ of below 70. In fact, this proportion seems to be closer to 1–1.5% (see REFS 1–3). (See REFS 89,90 for more on the controversial issues that surround the use of IQ tests, such as cultural components in IQ testing, differences in IQ between populations and the heritability of such differences.)

genetics. It is likely that this group of disorders could be caused by alterations in molecular pathways that are important for neuronal functions, especially those involved in cognitive mechanisms.

In recent years, striking progress has been made in identifying some of the X-linked genes associated with MR, providing important insights into some of the molecular and cellular dysfunctions that underlie it, such as those that affect chromatin remodelling and signal transduction. We review this progress and discuss the work that remains to be done. In addition, we consider current difficulties in applying some of these discoveries to diagnosing the cause of MR and to the genetic counselling of affected families.

X-linked mental retardation

Since 1890, many studies have reported that more males than females are present in the institutions and special schools that care for the mentally handicapped (reviewed in REF. 2). This was first thought to be due to societal biases, on the assumption that some affected girls might be kept at home. However, the finding of large families in which MR inheritance was clearly X linked, together with improved epidemiological studies, led to the gradual acceptance that this 20–30% male excess might be due to mutations in X-linked genes. During the late 1970s, the identification of the fragile X syndrome as a distinct clinical entity was an important step in this process. This syndrome is associated with physical and behavioural features that are present in most fragile X patients, and accounts for ~2–3% of MR in males and for ~1% in females (who are generally less affected than males)[7,8]. The interest in fragile X syndrome led to the description of other X-linked MR (XLMR) syndromes and of several large families in which MR is not associated with a specific clinical or metabolic phenotype (nonspecific or non-syndromic XLMR)[2].

Syndromic forms of XLMR are, in general, amenable to conventional positional-cloning strategies because families that share similar clinical phenotypes can be pooled for linkage analysis to narrow down a candidate interval. However, the situation is more complex for nonspecific XLMR as the first linkage studies of it showed extensive genetic heterogeneity; the linkage analysis of more than 70 families identified at least 10 non-overlapping regions that contain an X-linked MR (MRX) gene[9]. The candidate region for each family is very large, usually in the 20–30-cM range, and might

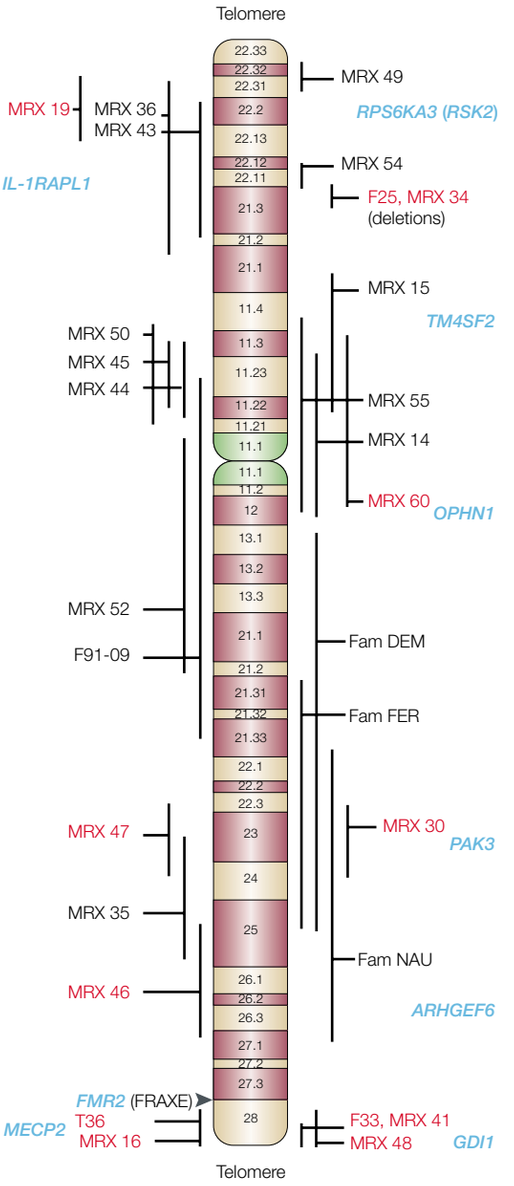

Figure 1 | **Genes and loci involved in nonspecific X-linked mental retardation.** Vertical bars indicate the candidate region for the mental retardation (MR) gene mutated in each of 25 (MRX) families studied by the European XLMR Consortium[42], with the addition of families MRX 19, MRX 30 and MRX 41, in which *RSK2*, *PAK3* and *GDI1* mutations were found. Families in which a mutation has been detected are marked in red, genes mutated in X-linked mental retardation disorders are shown in blue. *ARHGEF6*, Rac/Cdc42 guanine-exchange factor (GEF) 6; *FMR2*, fragile X mental retardation 2; *GDI1*, GDP dissociation inhibitor 1; *IL-1RAPL1*, interleukin-1 receptor accessory protein like; *MECP2*, methyl CpG-binding protein 2 (Rett syndrome); MRX, X-linked mental retardation; *OPHN1*, oligophrenin 1; *PAK3*, p21 (CDKN1A)-activated kinase 3; *RPS6KA3*, ribosomal protein S6 kinase, polypeptide 3; *TM4SF2*, transmembrane 4 superfamily member 2. F25, F33, F91–09, Fam DEM, Fam FER, Fam NAV and T36, are all family identifiers.

Table 1 | **Genes involved in syndromic forms of X-linked mental retardation***

Disease	Gene	Protein function	References
Syndromes with generally severe MR			
Adrenoleukodystrophy‡	*ABCD1*	Peroxisomal ABC transporter involved in catabolism of very long chain fatty acids	93
Coffin–Lowry syndrome	*RPS6KA3*	Serine/threonine protein kinase	24,26
Fragile X syndrome	*FMR1*	mRNA-binding protein, possibly controls mRNA transport and translation	7,8
Hunter disease (mucopolysaccharidosis type II)§	*IDS*	Iduronate sulphatase involved in mucopolysaccharide catabolism	94
X-linked hydrocephalus/ MASA syndrome	*L1CAM*	Adhesion molecule involved in neural cell interactions	95
Lesch–Nyhan disease	*HPRT*	Purine metabolism	96
X-linked lissencephaly (males); subcortical laminar heterotopia (females)	*DCX*	Microtubule-associated protein	97,98
Lowe oculocerebrorenal syndrome	*OCRL1*	Phosphoinositide phosphatase	99
Mental retardation and α-thalassaemia (X linked)	*ATRX*	DNA-binding helicase, involved in chromatin remodelling	11,12
Mohr–Tranebjaerg syndrome	*TIMM8A*	Mitochondrial protein imports proteins to mitochondrial inner membrane	100
Opitz G/ BBB	*MID1*	Ring box/B-box protein that associates with microtubules	101
Rett syndrome (females)	*MECP2*	Methyl CpG DNA-binding protein, and transcriptional repressor	32
Menkes disease (copper transport)‖	*ATP7A*	Copper-transporting P-type ATPase	102,103
OTC deficiency‖	*OTC*	Ornithine transcarbamylase	104
Pyruvate dehydrogenase deficiency‖	*PDHA1*	Pyruvate dehydrogenase	105
Syndromes with generally inconsistent and mild MR			
Aarskog-Scott	*FGD1*	Protein similar to Rho/Rac guanine-nucleotide exchange factors	106
Dyskeratosis congenita	*DKC1*	Protein that associates with small nucleolar RNA and human telomerase RNA	107
Simpson–Golabi–Behmel	*GPC3*	Heparane sulphate proteoglycan involved in the control of cell growth and division	108
Duchenne muscular dystrophy	*DMD*	Component of the large dystrophin-associated complex, which localizes to the sarcolemmal membrane; expressed in the brain	109
Incontinentia pigmenti (females)	*IKBKG*	NF-κB essential modulator (IKK subunit-γ)	110
Norrie disease	*NDP*	Secreted protein, acts as a growth factor?	111
Pelizaeus–Merzbacher	*PLP*	Principal protein component of myelin	112
Epilepsy with periventricular heterotopia	*FLN1*	Actin-binding protein	113

*Only those syndromes for which the gene has been identified are listed. For a list of other syndromes, see REFS 2,3. ‡In ALD, intelligence is normal until the onset of brain demyelination, which occurs during childhood in 40% of males carrying the mutation. §In this disease, patients with milder forms do not present with cognitive impairment. ‖In these three diseases, which often lead to early death of affected males, survivor males, patients with milder forms or some heterozygous female carriers show impaired cognitive function. *ABCD1,* ATP-binding cassette, subfamily D, member 1; *ATP7A*, ATPase, Cu^{2+}-transporting, α-polypeptide; *ATRX*, α-thalassaemia/mental retardation syndrome, X linked; *DCX*, doublecortex, lissencephaly, X linked (doublecortin); *TIMM8A*, translocase of inner mitochondrial membrane 8; *DKC1*, dyskeratosis congenita 1, dyskerin; *DMD*, dystrophin (muscular dystrophy); *FGD1*, faciogenital dysplasia; *FLN1*, filamin A, α lactin-binding-protein-280); *FMR1*, fragile X mental retardation 1; *GPC3*, glypican 3 ; *HPRT,* hypoxanthine phosphoribosyltransferase 1; *IDS*, iduronate 2-sulphatase; *IKBKG*, inhibitor of κ-light polypeptide gene enhancer in B cells, kinase-γ; IKK, IKappa B kinase; *L1CAM*, L1 cell-adhesion molecule; MASA, mental retardation, aphasia, shuffling gait and adducted thumbs syndrome, spastic paraplegia 1; MECP2, methyl CpG-binding protein 2; *MID1*, midline 1; *NDP*, Norrie disease (pseudoglioma); NF-κB, nuclear factor of κ-light polypeptide gene enhancer in B-cells inhibitor, α; *OCRL1*, oculocerebrorenal syndrome of Lowe; OTC, ornithine carbamoyltransferase; *PDHA1*, pyruvate dehydrogenase (lipoamide) alpha 1; *PLP*, proteolipid protein 1; *RPS6KA3*, ribosomal protein S6 kinase polypeptide 3.

contain 100–300 genes (FIG. 1). Linkage results cannot be pooled, even from families in which MRX genes map to overlapping regions, because such families might carry mutations in different genes.

Although the non-syndromic forms of XLMR are much more difficult for geneticists to study, progress towards identifying their underlying genetic causes has been made for these disorders and for syndromic XLMR. In the next section, we review recent progress in understanding three forms of syndromic XLMR.

Chromatin remodelling and XLMR

Genes have been identified for at least 20 XLMR diseases that have distinctive symptoms and varying degrees of MR (TABLE 1). Perhaps the most famous of these is the fragile X syndrome, which is the most common known cause of inherited MR and is associated with a chromosomal FRAGILE SITE in Xq27.3. The causative mutation is an expansion of a CGG trinucleotide repeat that disrupts the expression of the *FMR1* (fragile X mental retardation 1) gene[7,8] (FIG. 2). The FMR1 protein is an RNA-binding factor that is thought to regulate the transport or translation of specific mRNAs (for review, see REF. 10). Some of the other XLMR disorders are metabolic diseases (such as Lesch–Nyhan syndrome, adrenoleukodystrophy, mucopolysaccharidosis type II, Hunter syndrome and Menkes syndrome), or diseases that are associated with observable brain anomalies (such as X-linked HYDROCEPHALUS, X-linked LISSENCEPHALY and Pelizaeus–Merzbacher syndrome), and these are generally associated with prominent neurological manifestations (such as epileptic seizures or spasticity). Chromatin remodelling is a more recent addition to the cellular processes that are disrupted in syndromic XLMR. In this section, we review three disorders, the ATRX (for α-THALASSAEMIA mental retardation, X linked), Coffin–Lowry and Rett syndromes, which are associated with severe MR and in which chromatin remodelling is thought to be affected by mutations in three genes. Furthermore, mutations in these genes have subsequently been detected in some cases of nonspecific MR. There are also interesting differences between the syndromes in the way that they affect either sex: the ATRX syndrome affects exclusively males; Coffin–Lowry syndrome shows partial penetrance in females; and mutations in the *MECP2* (methyl CpG-binding protein 2) gene were initially detected in Rett syndrome — a disease that, according to its clinical definition (see below), affects only girls.

The ATRX syndrome. The ATRX syndrome is a very severe form of MR that is associated with biological signs of α-thalassaemia in red blood cells, and with characteristic facial features and genital anomalies[11,12]. The *ATRX* gene, identified in 1995, encodes a large nuclear protein that contains an amino-terminal zinc finger, a coiled-coil domain and seven helicase motifs that are also found in helicases of the SNF2/SWI2 protein family, which are involved in chromatin remodelling[11,13]. Most of the mutations in this gene are missense changes: truncating mutations (nonsense or frameshifts) are rare and are limited to either ends of the coding sequence, which indicates that most truncating mutations might lead to prenatal lethality. The identification of *ATRX* mutations in other rare clinical syndromes (such as Juberg–Marsidi (mental retardation, X linked, with growth retardation, deafness and microgenitalism), Carpenter–Waziri, Holmes-Gang and Smith–Fineman–Myers syndromes) provides an interesting example of SYNDROME LUMPING[14–16].

Although *ATRX* mutations are usually associated with severe MR (for example, speech is absent or severely limited in affected individuals), a family has recently been described in which two family members had the typical MR and facial features of ATRX, whereas two other members had mild MR with epilepsy and no facial dysmorphism[17]. Interestingly, the mutation in this family is a nonsense mutation at the amino terminus of the gene, which indicates that a downstream translation initiation codon might be used, allowing a partially functioning protein to be made. Females who carry an *ATRX* mutation are asymptomatic, probably because cells in which the normal X is active are selected for. Indeed, the mutated X chromosome is inactivated in all leukocytes of carrier females[11,18].

The ATRX protein associates with pericentromeric HETEROCHROMATIN and with the short arm of ACROCENTRIC chromosomes that contain ribosomal DNA arrays[19]. *In vitro* studies have shown that ATRX interacts and colocalizes with the HETEROCHROMATIN PROTEIN 1, and it has been reported to interact with another protein involved in chromatin remodelling, the homologue of the *Drosophila melanogaster* Enhancer of zeste[20–22]. Finally, alterations in the normal methylation patterns of ribosomal DNA clusters and of a Y-chromosome-specific repeat have been found in ATRX patients[23]. Although no obvious methylation differences were observed at the α-globin locus (which is downregulated in ATRX patients, as indicated by the signs of α-thalassaemia), it should be noted that this locus is very close to the telomere — a heterochromatic region — and so its expression might be influenced by the abnormal methylation or structure of heterochromatin in ATRX patients. The phenotype in ATRX syndrome might thus result from abnormalities in chromatin structure and in gene regulation at, or near, heterochromatic regions.

Coffin–Lowry syndrome. The Coffin–Lowry syndrome is characterized by severe MR, facial dysmorphism (which, in some cases, resembles that observed in ATRX patients) and progressive skeletal malformations. Other less-frequent manifestations of the syndrome include deafness and cardiac defects. The gene for Coffin–Lowry syndrome (*RSK2*, also called *RPS6KA3*) encodes a member of the RSK (90-kDa ribosomal protein S6 serine/threonine kinase) family of protein kinases[24]. *RSK2*, which maps to Xp22, is one of four RSK genes in humans. The recently identified *RSK4* gene is also X linked and is a good candidate for an MR gene because its deletion correlates with cognitive impairment in patients with contiguous gene syndromes caused by large Xq21 deletions. However, this hypothesis has yet to be confirmed as no point mutations in this gene have been found so far in XLMR families[25].

FRAGILE SITE
Chromosomal anomaly that appears as a region of decondensed or partially broken mitotic chromosomes under specific karyotyping conditions. The FRAXA and FRAXE fragile sites contain expanded CGG repeats that are methylated, affecting the expression of the *FMR1* and *FMR2* genes, respectively.

HYDROCEPHALUS
A condition, marked by the expansion of cerebral ventricles and by the compression of neural structures, that is caused by a block in the flow of cerebral spinal fluid or its overproduction.

LISSENCEPHALY
A brain malformation characterized by the incomplete development of the folds (gyri) of the outer region of the brain (the cerebral cortex), which causes the surface of the brain to appear abnormally thickened and unusually smooth.

THALASSAEMIA
Inherited disorders that are caused by the defective production of either the α- or β-globin chains of haemoglobin.

SNF/SWI
Chromatin-remodelling multiprotein complex initially identified genetically in yeast. Related complexes exist in mammals and are involved in the activation and repression of various genes.

SYNDROME LUMPING
When syndromes described under different names and with different clinical features are found to be caused by mutations in the same gene. Syndrome splitting is also common, when a clinical diagnosis that was thought to correspond to a single disease is shown to be caused by mutations in different genes.

HETEROCHROMATIN
Late-replicating, gene-sparse, condensed chromatin regions that are rich in repeated sequence.

ACROCENTRIC
When a centromere is close to one end of a chromosome.

HETEROCHROMATIN PROTEIN 1 (HP1). A protein that binds to highly repetitive, heterochromatic satellite DNA at centromeres and telomeres.

ENCEPHALOPATHY
A degenerative condition of the brain that can be caused by infectious disease, metabolic abnormalities, brain tumours, toxic drug effects or increased intercranial pressure.

The RSK2 protein has two ATP-binding kinase domains, regulatory phosphorylation sites and a docking site for the ERK kinase (a mitogen-activated protein kinase). At present, 86 mutations have been identified in 250 patients with a clinical phenotype indicative of Coffin–Lowry syndrome[26]. This low hit rate is probably due to the mis-classification of some patients, owing to the nonspecific nature of some of the clinical symptoms that define this syndrome. Mutations are heterogeneous and include truncating mutations (60% of cases) and missense mutations (38% of cases). Mutational analysis of *RSK2* showed that mutations in the gene are also associated with less severe forms of this syndrome, prompting a search for *RSK2* mutations in three families in which nonspecific XLMR maps to Xp22. In one family, a missense change was found near a regulatory phosphorylation site in RSK2. This mutation leads to an 80% reduction in the enzymatic activity of RSK2 and to a phenotype of mild MR, in the absence of other clinical features[27]. This residual RSK2 activity is sufficient to protect patients from severe MR and from skeletal abnormalities. However, this is likely to be an exceptional cause of nonspecific XLMR. In contrast to ATRX syndrome, Coffin–Lowry mutations do not lead to biased X inactivation and can therefore cause clinical manifestations in carrier females. Indeed, 10% of *RSK2* mutations have been identified in female patients with no affected male relatives, who have been ascertained through learning disabilities and through mild, but suggestive, facial and digital dysmorphologies[26].

The identification of the *RSK2* gene as the gene for Coffin–Lowry syndrome has also been useful for studies of the function of the gene. The high sequence similarity between the different human RSK proteins had made it difficult to assess their functional specificity, in terms of their upstream activators or downstream target genes. By analysing fibroblasts from Coffin–Lowry patients, it was found that RSK2 is required for the activation, by phosphorylation, of the transcription factor CREB, and for the induction of *FOS* expression, in response to epidermal growth factor (EGF) stimulation[28]. RSK2 is also necessary for EGF-induced phosphorylation of histone H3, an important event in chromatin remodelling[29]. Recently, the interaction between RSK2 and the CBP co-activator (CREB-binding protein, which is mutated in the MR disorder Rubinstein–Taybi syndrome) was shown to regulate histone H3 acetylation[30]. So, RSK2 seems to be important in chromatin-remodelling events and in gene regulation.

Rett syndrome. Rett syndrome is a severe disease that leads to the cessation and regression of psychomotor development in 1 in 12,000 girls and is associated with autistic manifestations. Commonly believed to be a dominant X-linked trait that causes prenatal lethality in boys, Rett syndrome has, for many years, been an enigma in human genetics research because its genetic analysis has been severely hampered by the fact that nearly all cases of the disease are sporadic. Nevertheless, the existence of rare families with two or more affected girls allowed the disease to be tentatively mapped to Xq28 in 1998 (REF. 31). The painstaking analysis of many genes in this region eventually led to the identification of mutations in a gene that had been known for many years, *MECP2*, which encodes a methyl CpG-binding protein[32]. Heterozygous *MECP2* mutations are found in 70–80% of girls who present with the characteristic features of Rett syndrome[33]. During the two years since the discovery of the involvement of this gene in the disease, *MECP2* mutations have been reported in more than 360 Rett patients (see, for instance, REFS 34,35). The relatively high frequency of the disease seems to be linked to the presence of several mutation hot spots: seven CpG-containing codons are implicated in 63% of the mutations, although there is no explanation for the high mutability of these seven codons. Furthermore, the carboxy-terminal coding region is a target for small deletions found in 10% of patients. Mutations that cause the typical Rett syndrome phenotype in girls lead to loss of MECP2 function, which affects, on average, 50% of a female patient's cells (as a result of random X-chromosome inactivation).

Once the Rett gene was identified, it was soon noticed that in some families with an affected girl, boys were born with a severe, and fatal, ENCEPHALOPATHY. Mutations in the *MECP2* gene were found in such cases, indicating that the phenotypic spectrum associated with *MECP2* mutations extends beyond the clinical definition of Rett syndrome. Indeed, *MECP2* mutations have also been found in families with nonspecific male MR[36,37], and a recent study has shown that *MECP2* mutations might account for 1–2% of males with MR (about 50% of the incidence of the fragile X syndrome)[38]. Mutations found in these MR males are different from those seen in females with Rett syndrome and probably cause a partial loss of MECP2 function.

MECP2 therefore represents the third XLMR gene that functions in chromatin remodelling (reviewed in REF. 39). The gene is widely expressed (most highly in the brain) and the particular sensitivity of neurons to its dysfunction remains a mystery. The recent conditional inactivation of *Mecp2* in the brains of mice gave rise to a postnatal neurological phenotype in heterozygous mutants that began at 5–6 weeks of age and that was reminiscent of certain Rett syndrome characteristics[40,41]. The results of these studies indicate that brain function, rather than brain development *per se*, is sensitive to the

Box 2 | **International consortia for studying mental retardation genes**

The European XLMR Consortium, created in 1996, comprises the groups of J.C. (Paris, France), J. P. Fryns (Leuven, Belgium), C. Moraine (Tours, France), H. van Bockhoven and B. C. J. Hamel (Nijmegen, The Netherlands), and H. H. Ropers (Berlin, Germany). The consortium has collected ~200 clinically well-characterized families with established or probable X-linked mental retardation. Significant linkage to a subchromosomal region of the X chromosome has been obtained for more than 40 families and cell lines have been established for most probands of each family. The Mendelian Cytogenetics Network, managed by N. Tommerup (Copenhagen, Denmark) collects information and cell lines on disease-associated balanced chromosomal rearrangements (not only those involving the X chromosome), and distributes probes for fluorescent *in situ* hybridization analysis to fine-map the translocation breakpoints[76].

Table 2 | **Genes involved in nonspecific X-linked mental retardation***

Gene (location)	Cloning strategy	Identified mutations‡	Frequency of mutations in MR	Potential function	References
FMR2/FRAXE (Xq28)	Fragile site and deletion	CGG expansion rare deletions	~0.2%	Transcription factor?	43,44
OPHN1 (Xq12)	Breakpoint cloning	1 PM	<0.5%	RhoGAP involved in regulating actin cyto-skeleton dynamics/ neuronal morpho-genesis	55
PAK3 (Xq22)	Candidate gene	2 PM	<0.5%	A Rac/Cdc42 effector involved in regulating actin cytoskeleton dynamics/neuronal morphogenesis	59,60
GDI1 (Xq28)	Candidate gene	3 PM	<1%	RabGDP-dissociation inhibitor involved in synaptic vesicle fusion, neuronal morphogenesis	51,52
IL-1RAPL1 (Xp21.3-22.1)	Deletion mapping	Rare deletions 1 PM	<0.5%	Unknown function, synaptic plasticity (?)	68
TM4SF2 (Xp11.4)	Breakpoint cloning	2 PM	<0.5%	A tetraspanin protein that interacts with integrins, and is is involved in regulating actin cytoskeleton dynamics (?)	64
ARHGEF6 (Xq26)	Breakpoint cloning	1 PM	<1%	Effector of Rho GTPases involved in regulating actin cytoskeleton dynamics/neuronal morphogenesis	61
MECP2 (Xq28)	Candidate gene (in male MR)	6 PM (missenses)	1–2% ?	Methyl CpG-binding protein	38

*The *RSK2* and *ATRX* genes are also involved in nonspecific MR in single families, mutations in these genes are otherwise associated with syndromic MR. ‡In independent families. *ARHGEF6*, Rac/Cdc42 guanine exchange factor (GEF) 6; *FMR2*, fragile X mental retardation 2; *GDI1*, GDP dissociation inhibitor 1; *IL-1RAPL1*, IL-1 receptor accessory protein like; *MECP2*, methyl CpG-binding protein 2; *OPHN1*, oligophrenin 1; *PAK3*, p21 (CDKN1A)-activated kinase 3; PM, point mutation; *TM4SF2*, transmembrane 4 superfamily member 2.

absence of Mecp2. This might be due either to general changes in transcription (for example, the incomplete repression of genes that are not normally expressed in neurons) or to the dysregulation of specific genes[33]. A comparative analysis of the transcriptome in wild-type and *Mecp2*-knockout mice might lead to a better understanding of Rett syndrome.

Nonspecific MRX genes

For a long time, identifying the genes that underlie nonspecific forms of XLMR seemed to be an almost impossible task, given the extensive genetic heterogeneity of this condition. However, progress in genome analysis and in the establishment of large collaborations between clinical and molecular research teams[42] (BOX 2) have led to considerable progress and to the identification of seven nonspecific MRX genes (eight including *MECP2*, TABLE 2). But there might be as many as 30 genes still to be found, as each identified gene accounts for only some of the MRX families that map to the region of the X chromosome that encompasses these MRX genes[9] (FIG. 1).

Two types of patient who carry X-chromosomal rearrangements have been particularly useful in studies of nonspecific XLMR. Patients with an X–autosome translocation (notably females in whom the normal X chromosome is inactivated, a well-known feature of such translocations) are useful because they will be deficient for an X-linked gene that lies at the translocation breakpoint. Males with microdeletions on the X chromosomes are also informative and are often detected by the presence of a contiguous gene syndrome, in which a known X-linked disease phenotype is combined with MR (in this case, the MR gene is expected to lie in the deleted region). In both cases, definitive proof that a gene is indeed an MR gene requires that point mutations or small intragenic deletions are identified in MRX families or at least in sporadic cases of MR in males.

Candidate gene strategies have also been fruitful in the search for nonspecific MRX genes. Mutation screens have focused on genes that are located in the appropriate region of the X chromosome in MRX families and that are known to be involved in neuronal development and function, or that are mutated in syndromic XLMR (such as *MECP2* or *RSK2*).

FMR2. Some mentally retarded individuals present with a fragile site in Xq28, but not with the CGG expansion in the *FMR1* gene that causes the fragile X syndrome. Such patients led to the identification of the *FMR2* gene, in which a CCG repeat is expanded and is associated with the FRAXE fragile site[43,44] (FIG. 2). Deletion of the *FMR2* gene has also been noted in one patient with developmental and speech delay. The expansion mutation in

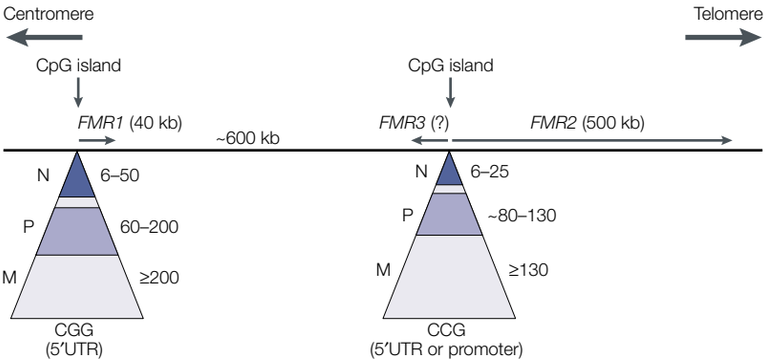

Figure 2 | **Expansion mutations at *FMR1* and *FMR2*.** The triplet-repeat range is indicated for normal alleles (N), for premutation alleles (P) and for full mutation, disease-causing alleles (M) of *FMR1* and *FMR2* (FMR, fragile X mental retardation). The CGG repeat is located in the 5′ untranslated region (UTR) in exon 1 of the *FMR1* gene; the exact location of the CCG repeat with respect to the transcription initiation site of the *FMR2* gene is still uncertain. Premutations do not lead to the abnormal methylation of the flanking CpG island (which controls the expression of the corresponding gene), but can expand to a full mutation upon maternal transmission. Full mutations are abnormally methylated, leading to the transcriptional repression of *FMR1* or *FMR2*. A small gene comprising two exons, called *FMR3*, which lies in the opposite orientation to *FMR2*, was recently identified; its expression is also abolished by an *FMR2* full mutation[91]. However, the significance of *FMR3*, which does not contain a long open reading frame, is unclear.

FMR2 is located 600 kb downstream of the *FMR1* gene and shares several properties with the *FMR1* mutation: the expanded repeat and the neighbouring CpG island are abnormally methylated, which suppresses *FMR2* transcription. Intermediate-sized expansions are unmethylated on the active X chromosome, are unstable upon transmission to offspring, and are therefore similar to fragile X PREMUTATIONS[45]. Male patients with the methylated expansion usually present with mild to borderline nonspecific MR, although individuals have been described who are either more severely affected or in the normal IQ range. The incidence of *FMR2* expansion is about ten times lower than that of the classic *FMR1* expansion[46]. *FMR2* encodes a nuclear protein of unknown function that is expressed in neurons and that belongs to a small group of DNA-binding proteins that might function as transcription factors[47,48].

Rab activation and αGDI. The Rab GTPases are a subgroup (of at least 40 members) of the small Ras-like GTPase family that are involved in neurotransmission[49]. In common with most small GTPases, Rab proteins cycle between an active GTP-bound and an inactive GDP-bound state through the action of regulatory proteins. GDP dissociation inhibitors (GDIs) are required to retrieve the GDP-bound form of Rab from the membrane and to maintain a pool of soluble Rab–GDP. In the mammalian brain, αGDI, encoded by the *GDI1* gene, is the most abundant form of GDI and regulates RAB3A and RAB3C — the Rab proteins that participate in synaptic vesicle fusion[50].

The mapping of *GDI1* to Xq28 made the gene an excellent candidate for XLMR in families showing linkage to this region, and *GDI1* mutations were found in three out of seven such families[51,52]. One is a nonsense mutation, whereas the two others are missense mutations that decrease the affinity between RAB3A and GDI.

Gdi1-deficient mice have shown a function for this protein in neurotransmitter release[53]. Furthermore, electrophysiological analysis of synaptic activity in the hippocampus of *Gdi1*-deficient mice has shown that they have an enhanced response to repetitive stimulation, a phenotype opposite to that of *Rab3a*-deficient mice[54]. Recent data reported by Ishizaki *et al.*[53] suggest that αGDI is important in suppressing the hyperexcitability of PYRAMIDAL NEURONS *in vivo*.

Three genes in the RhoGTPase pathway. Three of the newly identified MRX genes, *OPHN1* (oligophrenin 1), *PAK3* (p21 (CDKN1A)-activating kinase 3) and *ARHGEF6* (Rac/Cdc42 guanine exchange factor 6) encode proteins that interact with RhoGTPases, a family of small Ras-like GTPases that act in signal transduction pathways and that transduce extracellular-derived signals from the cell surface to the actin cytoskeleton of the cell and nucleus (FIG. 3).

OPHN1, which encodes oligophrenin, was found to be interrupted in a female patient carrying a balanced X;12 translocation associated with MR. A frameshift mutation that causes the premature termination of OPHN1 was then identified in one out of four MRX families showing linkage to the appropriate X-chromosome region[55]. *OPHN1* is predominantly expressed in fetal and adult brain, in both neurons and glial cells, and encodes a protein with similarity to Rho GTPase-activating protein (Rho GAP). Indeed, OPHN1 stimulates GTPase activity specifically for members of the Rho family of proteins, such as RhoA, Rac and Cdc42 (FIG. 3a). These proteins function in the organization of the cytoskeleton and, particularly, in growth-cone dynamics[56]. Rho GAP proteins increase the rate at which the GTP bound to Rho GTPases is hydrolysed, thereby helping to switch them off (FIG. 3b); loss of OPHN1 function might therefore result in constitutively active Rho proteins and altered actin cytoskeleton dynamics.

PAK3 is a member of the large family of p21-activating kinases (PAKs). It is highly expressed in the developing brain and acts as a Rac/Cdc42 downstream effector[57,58] (FIG. 3a). Mutation screening of this candidate gene identified a nonsense mutation in one MRX family[59], and a missense mutation that co-segregates with MR in another large family[60]. PAK proteins have been ascribed roles both in regulating actin cytoskeleton dynamics and in the Rac/Cdc42-induced activation of the mitogen-activated protein kinase cascades.

The *ARHGEF6* gene encodes a protein known as αPIX (or Cool-2) that has homology to the RhoGEFs (guanine nucleotide exchange factors for RhoGTPases) (FIG. 3b). Molecular analysis of an X;21 reciprocal translocation in a male with MR showed that this gene was disrupted by the rearrangement[61]. Mutation screening of 119 unrelated patients showed a single intronic mutation in all the affected males in a large MRX family. The mutation causes preferential exon

PREMUTATION
An unstable mutation that has no phenotypic effect but that is highly likely to mutate to a full mutation during transmission through the germ line, as is seen with some expanding trinucleotide repeats.

PYRAMIDAL NEURON
A class of neuron in the cerebral cortex with a pyramid-shaped cell body. These neurons send long axons down the spinal cord and form dendrites that extend laterally through the cortical layer that contains the cell body.

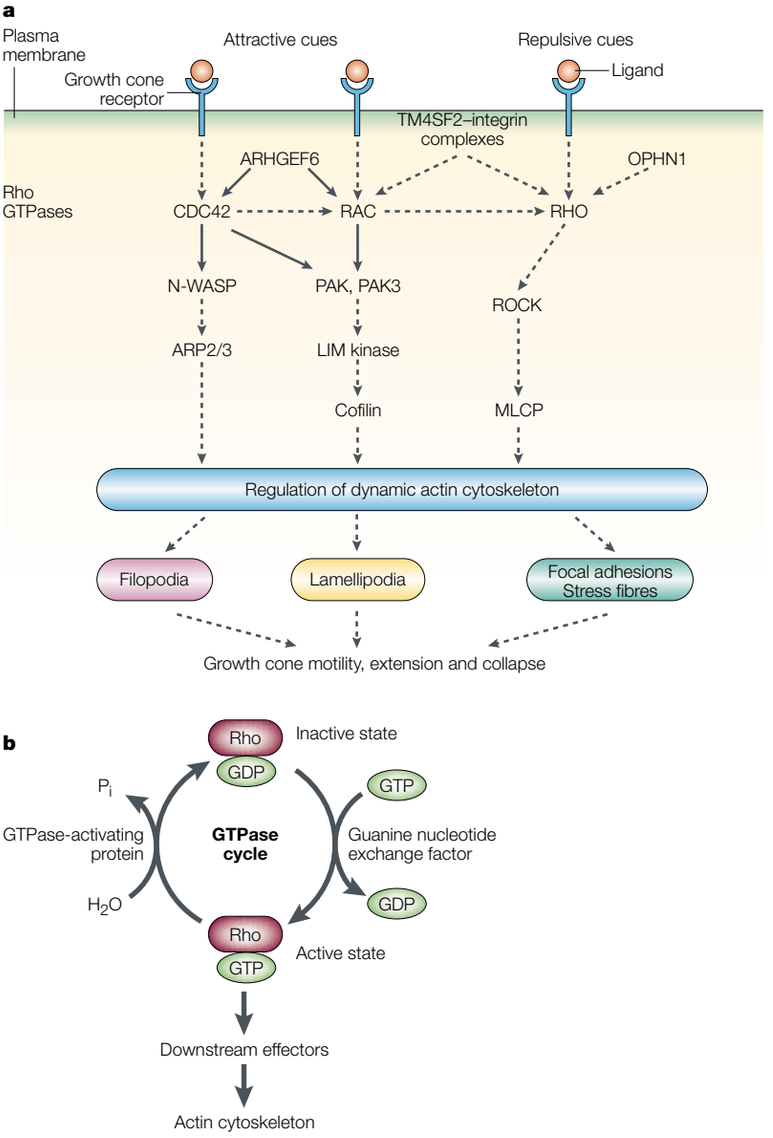

Figure 3 | **Signal transduction pathways and X-linked mental retardation.** **a** | Extracellular guidance cues interact with GROWTH CONE receptors, which in turn activate signalling cascades that involve Rho-like GTPases. Activated, GTP-bound, Rho GTPases stimulate FILOPODIA and lamellipodia formation or induce the collapse of the growth cones. The dysfunctioning of PAK3 (p21-activating kinase 3), OPHN1 (a RhoGAP), TM4SF2 (tetraspanin) and ARHGEF6 (a RhoGEF) is associated with mental retardation. (N-WASP, neuronal Wiskott–Aldrich syndrome protein; ARP2/3, actin-related protein 2/3.) The tetraspanin–integrin complexes can activate Rac and RhoGTPases through mechanisms reviewed by Giancotti[92]. Dotted arrows indicate simplified pathways, for which the protein partners involved are not fully identified (see also REFS 56,83). **b** | Control of RhoGTPases by GTPase-activating proteins (GAPs) and by guanine-nucleotide exchange factors (RhoGEFs). (Modified with permission from REF. 83. © (2000) Macmillan Magazines Ltd) (MLCP, myosin light-chain phoshatase; ROCK, Rho-associated kinase.)

skipping and the deletion of 28 amino acids[61]. The role of αPIX in brain development and neuronal morphogenesis remains to be addressed, but it is required for the recruitment of PAK to cytoskeletal actin-rich structures, such as focal complexes and LAMELLIPODIA, the formation of which is controlled by the activity of Cdc42 and Rac1 (REF. 62) (FIG. 3).

GROWTH CONE
The motile tip of the axon or dendrite of a growing nerve cell, which spreads out into a large cone-shaped appendage.

TM4SF2. The *TM4SF2* gene encodes a tetraspanin (also known as TALLA-1/T-cell acute lymphoblastic leukaemia antigen[63]), and is inactivated by an Xp11.4 breakpoint of an X;2 balanced translocation in a female patient with MR. Point mutations in *TM4SF2* have also been detected in 2 out of 33 XLMR families[64]. Tetraspanins are cell-surface proteins of 200–300 amino acids that span the membrane four times and form two extracellular loops. One of the key features of the tetraspanins is their ability to associate with one another and with β1-INTEGRINS, and class I and class II HLA proteins. Their interaction with β1-integrins might mediate diverse cellular processes, such as the regulation of actin cytoskeleton dynamics, the activation of signalling pathways, and cell proliferation, adhesion and migration[65]. Little is known about the role of tetraspanins in the physiology of the CNS, in which *TM4SF2* is highly expressed, notably in the cerebral cortex and hippocampus[64]. In *Drosophila*, the homologues of *TM4SF2* (encoded by the *late bloomer* gene) and of an α-integrin (Scab, also known as Volado) are involved in synapse formation and synaptic plasticity, respectively[66,67].

IL-1RAPL. IL-1RAPL (interleukin-1 receptor accessory protein-like) was identified by studies of a 350-kb deletion at Xp22.1-21.3 in an MRX family. The deletion overlapped with independent deletions in MR patients with contiguous gene syndromes that included the clinical features of X-linked glycerol kinase deficiency and adrenal hypoplasia[68]. Non-overlapping deletions and a nonsense mutation in this large gene were identified in patients with only cognitive impairment[68]. The homologous mouse gene is expressed in the developing and postnatal structures of the hippocampus, which is implicated in learning and memory[68]. The ligand(s) that induces the cascade in which IL-1RAPL is involved is still unknown.

MR and abnormal behaviour? The *MAOA* gene

During 1993, linkage analysis was reported in a Dutch family in which several males over successive generations had presented with mild or borderline MR, associated with aggressive or deviant behaviour[69]. The Xp11.3 region showed significant linkage to MR in this family, and when urine samples of affected family members were analysed, abnormal concentrations of monoamine metabolites were found[69]. This focused attention on two adjacent genes, *MAOA* and *MAOB*, in the candidate region, which encode monoamine oxidases A and B, respectively; enzymes that degrade the neurotransmitters serotonin and noradrenaline. A nonsense mutation was subsequently found in the *MAOA* gene in 'affected' males of this family[70]. Two years later, the report of aggressive behaviour in male mice that lack *Maoa* seemed to support the significance of these initial observations[71].

Despite numerous attempts to relate *MAOA* polymorphisms to aggressiveness, antisocial behaviour or anxious depressive alcoholism, no further human *MAOA* mutations have been reported[72–74]. In one study[74], 352 males with MR and 46 males attending

Table 3 | **Frequencies of chromosomal and X-linked MR in males**

Causes	Frequency* in male population	Frequency* in male MR patients
MR (IQ ≤ 70)	**1%**	
Chromosomal rearrangements	**1/600**	15%
Telomere deletions	(1/2,500–1/5,000)	2–4%
Fragile X syndrome	**1/4,000–1/5,000**	2–2.5%
MECP2 mutations	1/6,000–1/10,000	1–1.5%
Nonspecific XLMR		
30–50 genes, each gene	1/30,000–1/100,000	1/300–1/1000
	Σ1/1,000–1/2,000‡	Σ5–10%
Syndromic XLMR		
30 genes	Σ1/1,500–1/2,000‡	Σ5–7%
Total XLMR	1.3/1,000–2.1/1,000	13–21%

*The frequency values are approximate, apart from those in bold. The actual frequency of detection of telomere deletions or fragile X mutations in MR male patients depends on the extent of preselection based on clinical phenotype. ‡Σ, sum of frequencies. MR, mental retardation; *FMR1*, fragile X mental retardation 1; *MECP2*, methyl CpG-binding protein 2; XLMR, X-linked mental retardation.

a sexual disorder clinic were screened for *MAOA* mutations, but none were found. These results are unexpected because the *MAOA* coding sequence represents a mutation target comparable in size to that of other X-linked disease genes associated with a specific phenotype, for which hundreds of loss-of-function mutations have been described. How can one reconcile these contrasting observations? One possibility is that borderline intelligence with aggressive behaviour is a nonspecific phenotype that is influenced by many factors (genetic and environmental), which reduces the chances of finding a mutation in any specific gene. Furthermore, the institutionalized males with MR that have been studied[74] might not share the same phenotype of borderline MR that was observed in the Dutch family. Alternatively, the expression of the *MAOA* mutation was enhanced in the Dutch family by an undetected co-segregating functional variant in a nearby gene (possibly the *MAOB* gene). Whether *MAOA* mutations cause behavioural abnormalities and MR in other families is thus a question that remains to be resolved.

Prospects for autosomal MR genes

Although the excess of males with MR has focused interest on X-linked MR genes, autosomal genes are also likely to be involved in cognitive function and are therefore a target for further investigation. For autosomal-recessive forms, studying syndromic MR, such as the many forms of microcephaly[75], or large consanguineous families with nonspecific forms of MR might uncover autosomal genes associated with such disorders. However, tackling the genetic basis of autosomal-dominant MR will be much harder because severe dominant MR only occurs through new mutations or when there is a highly variable phenotype. These situations render linkage analysis almost impossible in the absence of other specific clinical features. Even genes at translocation breakpoints[76], or in large deletions associated with MR, might be difficult to validate by identifying mutations in patients without such rearrangements. Nevertheless, recent findings have shown that telomeric chromosomal rearrangements (unbalanced translocations or deletions) might account for 5–7% of severe MR that is generally associated with dysmorphic features or other clinical manifestations[6]. Up to 50% of these rearrangements, which cause monosomy or trisomy of gene-rich subtelomeric regions, are inherited from a clinically normal parent carrying the balanced translocation, and thus confer a high recurrence risk of MR in the family.

Diagnostic implications

Identifying causative mutations in MR genes is important for diagnosing individuals suspected of having a genetic form of mental retardation (in the absence of unambiguous clinical criteria) and for genetic counselling purposes. Testing for the fragile X expansion mutation is thus commonly done in MR cases, with generally little clinical preselection, and with a consequently low hit rate for positive diagnoses (~2–5%). This test also misses point mutations in the *FMR1* gene that would result in the same clinical outcome.

For XLMR syndromes, such as Coffin–Lowry or ATRX, the situation is more difficult because mutations are diverse and can occur almost anywhere in these large genes, requiring that they be screened exon by exon. The cost of testing is such that patients have to be selected by a competent clinical geneticist to reach a reasonable hit rate for positive diagnoses (~35% for Coffin–Lowry syndrome)[26]. But such clinical screening will miss cases that do not fit the main clinical criteria for the disease. For example, girls diagnosed using strict clinical criteria for Rett syndrome have an 80–90% chance of being the carriers of an *MECP2* mutation. However, *MECP2* mutations are also found at a lower frequency in girls with atypical Rett syndrome (patients who meet only three of the seven main diagnostic criteria for the disease)[77], and also in a few per cent of males with very different phenotypes (either congenital encephalopathy, which leads to neonatal death, or moderate mental retardation)[36–38,78]. Protein-based tests (especially if they test for both protein presence and function) might provide an alternative to mutation screening, if the target proteins are present in easily sampled tissues (such as blood, hair or buccal epithelium)[79,80].

At present, sporadic cases of male MR are not screened for the known nonspecific MRX gene mutations for technical and economic reasons. The average X-linked disease has an incidence of ~1 in 30,000–100,000 males (an incidence about tenfold lower than that of the fragile X syndrome), and a similar frequency is likely for most MRX genes. The higher incidences of other X-linked disorders, such as Duchenne muscular dystrophy or Rett syndrome, are accounted for by a large gene and/or by the presence of mutation hot spots. Given a 1% incidence of MR in males, the chance of observing a mutation in a given gene, in a sporadic MR male patient, will be 1–3 in 1,000 (TABLE 3). Given the average gene size of 15–20 exons, this translates into one mutation being found for every 5,000–10,000 exons tested. It is highly unlikely, given the present technologies, that a laboratory would

FILOPODIA
Long, thin finger-like exploratory cell extensions found in crawling cells and growth cones.

LAMELLIPODIA
Thin, sheet-like cell extensions found at the leading edge of crawling cells or growth cones.

INTEGRINS
Transmembrane proteins that function as heterodimers and are involved in cell–cell and cell–extracellular-matrix interactions.

engage in such a search. Even if the search is limited to probable X-linked cases, the efficiency would probably not increase by more than 5–10-fold. This explains why mutations in nonspecific MR genes have been found up to now in only 2–3 families, except for the expansion mutation in *FMR2* (TABLE 2). This also accounts for the striking deficit in *FMR1* point mutations (only three have been reported), as the chances of observing such a mutation is low[81], owing to the relatively poor specificity of the clinical phenotype that results from loss of FMR1 function. This contrasts with the 367 mutations reported for the *MECP2* gene in Rett syndrome patients in less than two years[34,35]. If the 2% incidence of *MECP2* missense mutations in male MR[38] is confirmed by further studies, this gene will become an important diagnostic target for male MR, especially as it is a relatively small gene.

Conclusion

The genetic complexity that underlies cognitive functions seems to be enormous. Recent advances in finding MR genes indicate that processes involved in the establishment, stabilization and remodelling of connections between neuronal cells are important in mental retardation disorders[82,83]. (It is noteworthy that dendritic spine morphology is reported to be abnormal in patients with fragile X syndrome and in the *Fmr1*-knockout mouse[84].) Delineating the monogenic causes of MR, and their molecular and cellular consequences, will provide insight into the mechanisms that are required for the normal development of cognitive functions in humans.

Analysis of the X-chromosome sequence, once completed, and high-throughput sequencing in MR patients should lead to the identification of most MRX genes in the future. How this can be translated into diagnostic tests that will provide answers to parents of children with MR about its causes, the risk of its reoccurrence in the family and the possibility of prenatal diagnosis, is an open question. Methodological improvements in mutation screening might help to overcome this problem, but some, such as the use of oligonucleotide arrays for mutation detection, are at present prohibitively expensive, and many arrays would be required to cover the full range of MRX genes[85]. Protein-based assays in an array format might provide an alternative test[86], in some cases, for genes that are expressed in cells that can be easily sampled. Meanwhile, a coordinated effort to sequence MRX genes in families with definite or possible XLMR might identify mutation hot spots, which could provide a first target for screening in MR patients.

Finally, it should be stressed that genetic counselling and prenatal diagnosis related to mental handicap raise sensitive ethical issues, especially for the milder forms of MR (such as for FRAXE, or for carrier females of a full fragile X mutation). Assessing cognitive function is complex, and performance can be subject to profound social and environmental factors in the family and in schools. The level of expectation with respect to intellectual performance also depends on the family, and on the type of society to which an individual belongs. Great care should thus be exercised in the diagnostic and genetic counselling applications of this fascinating research domain.

Links

DATABASE LINKS **Smith–Lemli–Opitz | Duchenne muscular dystrophy | Prader–Willi | Angelman | Williams–Beuren | fragile X | *FMR1* | Lesch–Nyhan | adrenoleukodystrophy | mucopolysaccharidosis type II | Menkes | Pelizaeus–Merzbacher | ATRX | Coffin–Lowry | Rett | *MECP2* | *ATRX* | Juberg–Marsidi | Smith–Fineman–Myers | Enhancer of zeste | *RSK2* | *RSK4* | ERK kinase | *FOS* | EGF | CBP co-activator | Rubinstein–Taybi | *FMR2* | Rab | *GDI1* | RAB3A | *Gdi1* | *Rab3a* | *OPHN1* | *PAK3* | *ARHGEF6* | RhoA | Cdc42 | *TM4SF2* | *late bloomer* | Scab | *MAOA* | *MAOB* | *Fmr1***

FURTHER INFORMATION **Wechsler intelligence scale | Stanford Binet intelligence scale | XLMR Genes Update web site | Jean-Louis Mandel's lab**

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