1 INTRODUCTION

1.1 Identifying genetic causes of mental retardation: historical overview

In studies on the behavioural and genetic aspects of cognitive disorders, two important terms, namely intelligence quotient (IQ) and mental retardation (MR), have been adopted by the scientific community, and are therefore used frequently in this dissertation. IQ is the result of a standardised test, typically the Wechsler's Scale (Wechsler 1974), which is designed to measure intellectual ability. Such tests have obvious limitations, but they enable a semi-standardised characterisation of mental disability, which facilitates scientific communication. Within this study and related research, MR is defined by an IQ of less than 70, in combination with problems in adaptive behaviour. It has multiple causes, including environmental factors, chromosome aneuploidies, and specific gene defects. An estimated 2-3% of the population is affected (Chelly and Mandel 2001). Although the aetiology of MR is complex, the predominance of males suffering from the disorder, and the relative ease of dissecting X chromosome genetics provided the starting point for recent investigations into the genetic factors important for cognitive development. With the identification of several families exhibiting X-linked mental retardation (XLMR), and the establishment of several international collaborations such that clinical and genetic information from these families could be shared, advances on the X chromosome have confirmed that there are numerous specific monogenic forms of MR. Of significant historical importance is the recognition of Fragile X syndrome (FRAXA), and the identification of the fragile site mental retardation 1 (*FMR1*) gene. Fragile X syndrome is caused by a CGG repeat expansion in the *FMR1* 5' untranslated region, which is then abnormally methylated. With a prevalence of 1 in 4000 males and 1 in 7000 females, this is the most common monogenic form of mental retardation known at present (for review, see Crawford et al. 2001).

The spectrum of disorders for which MR is a feature includes a broad range of distinct genetic entities; XLMR has therefore been divided into two sub-groups: syndromic XLMR (MRXS), which includes FRAXA and other MR-associated disorders that can be defined by a set of specific clinical features, and non-syndromic XLMR (MRX), which includes all X-linked forms of MR for which the only consistent clinical feature is mental retardation. In the last fifteen years, the genetic defects responsible for many MR-associated syndromes have been identified. These X-linked syndromes and associated genes are listed in Table 1. This

summary highlights the fact that defects in diverse biochemical processes, such as intracellular signalling and transcriptional regulation, as well as cellular metabolism and neuronal migration, converge on a mental retardation phenotype. Recent advances in our understanding of the genetics of non-syndromic mental retardation likewise reflect this heterogeneity (MRX genes are also listed in Table 1, indicated with *). Functional characterisation of these proteins has shed light on molecular mechanisms important for brain function, thereby illuminating trends that were not immediately apparent, namely the involvement of specific aspects of both G protein signalling and chromatin remodelling in MR.

Table 1: X-chromosomal genes associated with MR

GENE	DISORDER	PROTEIN FUNCTION	OMIM #
NLGN4	Autism	Neuroligin family of synaptic plasma membrane proteins	300427
MID1	Opitz syndrome	Microtubule-associated gene involved in cell proliferation and development	300000
PRPS2	PRPS2 deficiency	Phosphoribosylpyrophosphate synthetase	311860
OFD1	Oro facial digital	Unknown	300170
STK9	West syndrome	Serine threonine protein kinase, cyclin dependent kinase-related	300203
PDHA1	PDH deficiency	Pyruvate dehydrogenase subunit	312170
**RSK2/ RPS6KA3*	Coffin-Lowry syndrome/MRX	Serine-threonine protein kinase	300075
**ARX*	West Syndrome/MRX	*Aristaless*-related homeobox gene	300382
**IL1RAPL*	MRX	Interleukin 1 receptor accessory protein-related	300206
GK1	Hyperglycerolemia	Glycerol kinase	307030
DMD	Duchenne Muscular Dystrophy	Dystrophin protein with spectrin repeats and zinc finger domain	300377
OTC	Ornithine decarboxylase deficiency	Ornithine decarboxylase	311250
**TM4SF2*	MRX	Tetraspanin family of transmembrane proteins	300096
MAO-A	Brunner syndrome (mild MR)	Monoamine oxidase	309850
NDP	Norrie disease	Cystine-knot domain containing protein	310600
**ZNF41*	[1]MRX	Zinc finger gene involved in transcriptional repression	314995
**PQBP1*	[2]MRX	Polyglutamine binding protein	NA
**FGD1*	Faciogenital dysplasia/MRX	Harbours Rho/Rac GEF homology domain	305400
**OPHN1*	Cerebellar ataxia/MRX	Rho-GTPase-activating protein	300127
NLGN3	Autism	Neuroligin family of synaptic plasma membrane proteins	300336
ATRX/ XNP	X-linked α-thalassemia and mental retardation syndrome	Helicase family member with role in transcriptional regulation	300032
ATP7A	Menkes disease	ATPase family member with role in copper transport	300011
PGK1	PGK deficiency	Phosphoglycerate kinase 1, glycolysis enzyme	311800
TIMM8A	Mohr-Tranebjaerg syndrome	Translocase of the inner mitochondrial membrane	300356

GENE	DISORDER	PROTEIN DESCRIPTION	OMIM #
PLP	Perlizaeus-Merzbacher	Proteolipid protein, major myelin constituent	300401
PRPS1	PRPS1 deficiency	Phosphoribosylpyrophosphate synthetase	311850
**FACL4*	MRX	Long chain fatty acid CoA ligase	300157
**PAK3*	MRX	p21-activated protein kinase	300142
DCX	X-linked lissencephaly (males) and subcortical laminar heterotopia (females)	Doublecortin gene involved in neuronal migration, may be involved in Ca2+ signalling	300121
**AGTR2*	MRX	Angiotensin II receptor, type 2	300034
OCRL1	Lowe oculocerebrorenal syndrome	Lipid phosphatase involved in phosphatidylinositol 4,5-bisphosphate regulation	309000
GPC3	SGB syndrome	Glypican 3, cell-surface proteoglycan	300037
PHF6	BFL syndrome	Plant homeodomain (PHD)-like zinc finger-related protein	300414
HPRT	Lesch-Nyhan syndrome	Hypoxanthine (guanine) phosphoribosyltransferase 1	308000
**ARHGEF6*	MRX	Rho guanine nucleotide exchange factor 6	300267
SOX3	MR and growth hormone deficiency	DNA-binding protein involved in transcriptional regulation	313430
FMR1	Fragile X syndrome (with fragile site FRAXA)	RNA-binding protein with putative function in regulation of translation	309550
**FMR2*	MRX (with fragile site FRAXE)	Nuclear protein with putative transcription transactivation potential	309548
IDS	Hunter syndrome	Iduronate 2 sulfatase, involved in polysaccharide metabolism	309900
SLC6A8	Epilepsy and dysmorphism/MRX	Solute carrier 6, neurotransmitter transporter, creatine	300036
ABCD1	Adrenoleukodystrophy	ATP-binding cassette (ABC) transporter superfamily, involved in catabolism of long chain fatty acids	300371
L1CAM	X-linked hydrocephalus, MASA syndrome	Neural cell adhesion molecule, integral membrane glycoprotein	308840
**MECP2*	Rett syndrome/MRX	Methyl CpG binding protein involved in transcriptional repression	300005
FLNA	Heterotopia, X-linked dominant	Filamin 1, involved in anchoring of membrane proteins for the actin cytoskeleton	300017
**GDI1*	MRX	Rab family GDP dissociation inhibitor	300104
IKBKG/ NEMO	Incontinentia pigmenti	NF-kappa-B essential modulator	300248
DKC1	Dyskeratosis congenita	Predicted role in cell cycle and nucleolar function	300126

Genes associated with chromatin remodelling in red
Genes associated with Rho-GTPase signalling in blue
Genes implicated in non-syndromic MRX indicated with *
Genes are listed according to chromosome location, from Xp telomere to Xq telomere

References:

Chelly and Mandel 2001
XLMR Update (October 2003 Petro Chiurazzi): http://xlmr.interfree.it/home.htm
Online Mendelian Inheritance in Man™ (OMIM™): http://www.ncbi.nlm.nih.gov/Omim
[1]Shoichet et al. 2003
[2]Kalscheuer et al. 2003

Also of significance is the recent observation that the boundary between syndromic and non-syndromic MR is not always clear. For example, the recent discovery that mutations in *ARX*, the human homologue of the Drosophila gene *Aristaless*, are responsible for syndromic MRX with infantile spasms, Partington syndrome, and also non-syndromic MRX (Bienvenu et al. 2002; Stromme et al. 2002) clearly illustrates that mutations in a single disease gene may result in a variety of different, clinically distinguishable disorders. This has been observed for an increasing number of genes implicated in both MRXS and MRX, including *MECP2* (Amir et al. 1999; Couvert et al. 2001), *AGTR2* (Vervoort et al. 2002), *FGD1* (Pasteris et al. 1994; Lebel et al. 2002), *RSK2* (Trivier et al. 1996; Hanauer and Young 2002), *ATRX* (Villard et al. 1996b; Gibbons et al. 2000), and *L1CAM* (Jouet et al. 1994; Brewer et al. 1996). Studies on *L1CAM* mutations in a family with a variety of neurological abnormalities (Fryns et al. 1991; Ruiz et al. 1995) highlighted the fact that a specific mutation can even result in widely variable phenotypes within a single family.

Given this increasingly visible overlap in the underlying molecular causes of non-syndromic and syndromic XLMR, studies on the molecular aspects of these disorders can be combined to construct a more general understanding of the molecular basis of cognitive function, and within this framework, candidate gene searches can extend beyond the X chromosome. Indeed, this is now feasible. Two autosomal genes for mental retardation have already been identified: the neurotrypsin gene *PRSS12* on chromosome 4 (Molinari et al. 2002), and the Rho-GTPase activating protein MEGAP/srGAP3 on chromosome 3 (Endris et al. 2002). Another autosomal gene, the LIM domain kinase gene *LIMK1*, is linked to MR through its association with the visuo-spacial cognitive deficits of Williams syndrome (Frangiskakis et al. 1996).

The functional links between known MRX genes have provided the framework for ongoing investigations into the molecular basis of cognition, which now include analysis of both X-chromosomal and autosomal genes.

1.2 Mechanisms by which gene defects cause MR

The mechanism by which specific gene defects play a causal role in mental retardation is particularly clear in those diseases associated with macro-anatomical brain malformations. As in the case of brain defects resulting from trauma, structural abnormalities arising from developmental errors also have an impact on the intricate neuronal networks required for normal information processing. X-linked lissencephaly, for example, also known as 'smooth brain', is associated with loss of complexity in the cerebral cortex, resulting from neuronal migration defects. It is caused by mutations in the doublecortin gene (*DCX*), which is expressed exclusively in foetal brain (des Portes et al. 1998; Gleeson et al. 1998). A second disorder that is likewise associated with significant morphological defects is X-linked hydrocephalus. The clinical characteristics include enlarged cerebral ventricles and mental retardation, and there is often associated spastic paraparesis. It has been shown that the disorder results from mutations in the L1 cell adhesion molecule *L1CAM* (Rosenthal et al. 1992). L1CAM is an integral membrane glycoprotein with an established role in the central nervous system (CNS), specifically in cell adhesion and neurite outgrowth (Kenwrick et al. 2000). In both of these syndromes, it is plausible that the cognitive disability is a secondary effect resulting from substantial alterations in brain anatomy.

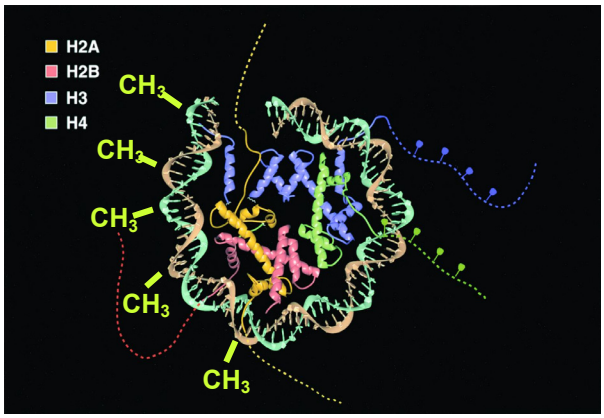
A second subgroup of mental retardation consists of those forms that are associated with metabolic defects. Given the high metabolic requirements of the brain, it is not surprising that gene defects directly affecting enzymes necessary for breakdown of fuel into non-toxic metabolites can have observable effects on mental function. Perhaps the best known example of this phenomenon is the developmental and cognitive disability associated with phenylketonuria, which results from a deficiency of phenylalanine hydroxylase and build up of toxic phenylalanine (for review, see Zschocke 2003). Similarly, ornithine transcarbamylase (OTC) deficiency, and pyruvate dehydrogenase (PDH) deficiency, caused by mutations OTC and PDHA1 (Rozen et al. 1985; Brown et al. 1994), respectively, are associated with mental retardation. An additional related example is provided by X-linked adrenoleukodystrophy, which is caused by mutations in the ATP-binding cassette transporter gene *ABCD1* and associated with defective catabolism of long chain fatty acids. While this disorder is phenotypically variable, it sometimes presents as behaviour disorders and learning difficulties in young children (Gartner et al. 1998).

For most non-specific forms of mental retardation identified so far, the causative gene defects identified do not fall into one of these two categories. The link between disease gene and disease mechanism is often much more obscure. Recently, however, and of particular relevance to this study, two other general mechanisms have been linked to cognition. In particular, the association of the methyl CpG binding protein MeCP2 with both Rett syndrome and non-specific mental retardation (Amir et al. 1999; Couvert et al. 2001) has implicated transcriptional repression, through regulation of chromatin architecture, specifically in brain development and function. This concept has since been corroborated by studies that connect other MRX genes to general regulation of transcription. The other, and perhaps most significant trend identified to date is the involvement of defective GTPase-mediated signalling cascades in mental disorders. Several known MRX genes, as indicated in Table 1, are key components of these processes, which are introduced in some detail in the next two sections.

1.2.1 Chromatin remodelling and MR

Correct regulation of gene expression is a complex process, which requires both the activity of specific transcription factors and intricate regulation of chromosome structure. It has been long since established that transcriptionally active regions are associated with regions of less densely packed chromatin, whereas more densely packed chromatin is typically transcriptionally inactive. The mechanisms governing this regulation, however, are only recently being uncovered. Chromatin, made up predominantly of chromosomal DNA and histone proteins, is packaged tightly into a series of coiled structures called nucleosomes. Each nucleosome (depicted in detail in Figure 1) consists of a histone octamer made up of

Figure 1: Structure of the nucleosome
Diagram of a nucleosome, showing the histone octamer with two of each of four histone proteins (H2A, H2B, H3 and H4), around which the DNA double helix is coiled. Protruding histone tails can be acetylated (see H3 and H4, as indicated with blue and green dots). Alternatively, methylation of DNA (indicated by CH_3 in yellow) leads to recruitment of histone deacetylases and hypoacetylation of histone tails (see H2A and H2B with tails that are not acetylated).



Adapted from Kornberg and Lorch 1999

two of each histone (H) 2A, 2B, 3, and 4, with the DNA double helix wound around its circumference. Each histone protein has two domains: a central structure, lying within the external DNA ring, and an amino terminal tail that extends beyond the coil structure and is free to interact with proteins that influence nucleosome organisation. In particular, these tails can be modified by histone acetyltransferases (HATs), resulting in either histone hyperacetylation, which is associated with transcriptional activation, or histone hypoacetylation, which is correspondingly associated with transcriptional repression (Allfrey 1977). It is widely accepted that histone acetylation results in a more open structure of the local chromatin, allowing for basal transcriptional machinery to access the promoter regions in DNA, thereby activating transcription, whereas hypoacetylation has the reverse effect. Another relevant point is the understanding that DNA hypermethylation of promoter-associated CpG islands results in transcriptional repression. This phenomenon is critically important for both imprinting and X-inactivation, which rely on transcriptional silencing of entire regions of the genome. While several steps are involved in each of these two processes, it has been shown that both involve DNA hypermethylation. In the case of imprinting, DNA methyltransferases are required for initiation of silencing; in X-inactivation, maintenance of gene silencing relies on methylation of specific promoter regions (Jaenisch and Bird 2003).

Histone deacetylation and DNA methylation have a long association with transcriptional repression, but the molecular link between the two and their involvement in neurological disorders has only recently been highlighted. This link was first shown in studies on the methyl binding domain (MBD) protein MeCP2, mutations in which were later implicated in the aetiology of both Rett syndrome and non-specific mental retardation. Rett syndrome is a neurological disorder characterised by regression of psychomotor development and typically associated with mental retardation and/or autistic symptoms. It affects predominantly girls; for reasons that are at present poorly understood, non-lethal *MECP2* mutations in boys result in a much less specific mental retardation phenotype (Hammer et al. 2002). Functional MeCP2 binds specifically to hypermethylated DNA, and represses transcription by recruiting protein complexes that contain both histone deacetylases (HDACs) 1 and 2 via its interaction with the transcriptional corepressor Sin3A (Nan et al. 1998). This interaction facilitates the deacetylation of histones in specific methylated chromosome regions, enabling the dense packaging of chromatin that prevents transcription factor binding and activation of local genes.

At least two other proteins are likewise associated with both chromatin remodelling and mental disability. The ATRX protein, implicated in X-linked α-thalassemia and mental retardation syndrome, is a member of the SNF2 family of helicase/ATPases, which play an established role as modifiers of chromatin structure. Additionally, ATRX contains a highly conserved plant homeodomain (PHD) motif that is present in many chromatin-associated proteins. Moreover, *ATRX* mutations are associated with diverse alterations in patterns of global DNA methylation (Gibbons et al. 2000), affirming the links between DNA methylation, chromatin remodelling, and mental retardation that became apparent upon analysis of MeCP2 dysfunction. The relationships between these phenomena are depicted in the schematic diagram in Figure 2. Although the precise mechanism by which associated gene defects lead to cognitive disabilities has yet to be elucidated, the involvement of MRX

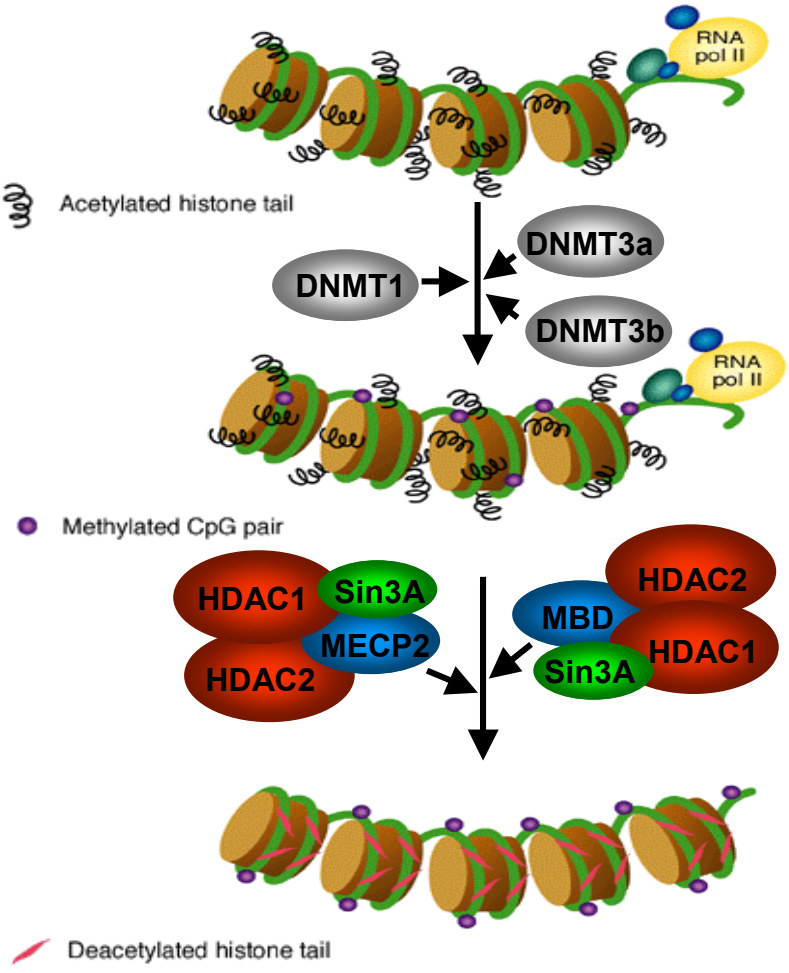


Figure 2: Links between DNA methylation, histone acetylation and transcriptional repression
The nucleosomes depicted above represent transcriptionally active genomic regions. Histones are acetylated (indicated by external tails in black), resulting in an open conformation that allows access to DNA by the basic transcription machinery (represented here by RNA pol II in yellow). Activity of DNA methyltransferases (DNMTs) leads to methylation of CpG dinucleotides (indicated in violet). Following methylation, methyl CpG binding proteins (MBDs such as MeCP2) recruit large transcriptional repressor complexes harbouring histone deacetylases (HDACs) and the Sin3A transcriptional co-repressor. Histones are then deacetylated (deacetylated tails are indicated in red below), which results in a closed conformation that does not allow the basic transcription machinery to access the DNA.

Adapted from Strathdee and Brown 2000

genes in the regulation of chromatin structure highlights the importance of this process specifically in neurological development.

1.2.2 GTP-binding proteins, signal transduction and MR

Many processes are regulated by extracellular signals that are transmitted from the plasma membrane to the nucleus through various molecular cascades. For example, signal transduction via the Ras superfamily of small GTP-binding proteins is one critical pathway by which hormone signals reach their final effector molecules. In response to ligand-receptor interactions at the cell surface, these proteins bind to GTP on the cytosolic side of the cell membrane and activate a cascade of downstream signals. With regard to this investigation, relevant studies that link specific signalling cascades to mental disability are those that involve the Rab-GTPase and the Rho-GTPase subfamilies of GTP-binding proteins. These proteins act as molecular switches for signal transduction by oscillating between an active, GTP-bound state, which initiates downstream cascades, and an inactive GDP-bound state. The intrinsic GTPase activity of Rho/Rab proteins, stimulated by GTPase activating proteins (GAPs), mediates the hydrolysis of GTP to GDP, thereby inactivating the signal. Guanine nucleotide exchange factors (GEFs), on the other hand, act as positive signal regulators through replacement of GDP by GTP, resulting in the active conformation. A third important regulatory function is served by the guanine dissociation inhibitors (GDIs), which maintain these GTPases in their GDP-bound state, and thereby prevent cycling into the active, GTP-bound state. Like the GAPs, therefore, GDIs negatively regulate signalling through the GTPases. These relationships are depicted in Figure 3. The oscillation between the active and inactive state of the GTPase-mediated signal relies on a balance of activity of the GAPs, GEFs, and GDIs.

It is striking that three out of fourteen genes that have been implicated in non-syndromic forms of X-linked mental retardation are directly linked to basic elements of this fundamental regulatory cycle. Mutations in the guanine dissociation inhibitor *GDI1*, for example, cause the mental retardation phenotype in two MRX families (D'Adamo et al. 1998). OPHN1, which harbours a Rho-GAP motif and has been shown to stimulate the GTPase activity of the Rho-GTPase family members RhoA, Rac1, and Cdc42 has also been implicated in mental retardation (Billuart et al. 1998). Mutations are associated with loss of function of OPHN1, suggesting that hyperactivation of the Rho-GTPase signallosome may

play a causal role in cognitive dysfunction. Mutations in the guanine nucleotide exchange factor *ARHGEF6*, which likewise mediates GTP-GDP cycling of Rho-GTPases, are also associated with mental dysfunction (Kutsche et al. 2000). Additionally, the Rho-GTPase activating gene *MEGAP/srGAP3*, a close relative of *OPHN1*, likely plays a critical role in mental function. Endris et al. (2002) showed that this gene is affected in several unrelated patients with MR-associated disorders. Together, these four examples clearly implicate GTP signalling in cognition, and the confirmed association of OPHN1, ARGHEF6, and MEGAP/srGAP3 with the Rho subfamily of small GTPases suggests that this particular pathway may be especially important. Studies involving effectors of Rho-

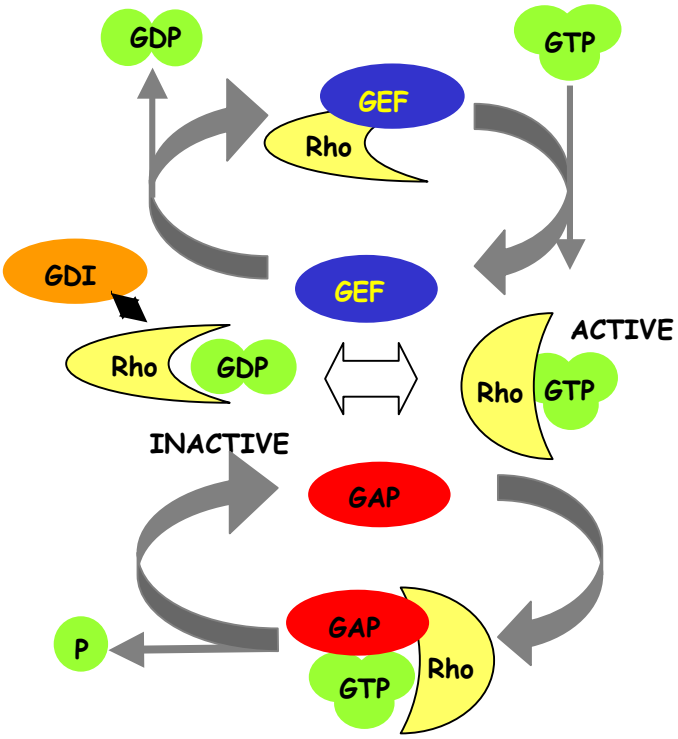


Figure 3: Regulation of signalling through Rho-GTPases
Extracellular signals are mediated in part by GTP signalling. This diagram depicts the cycling of the Rho subfamily of small GTPases (Rho, yellow) between an inactive GDP-bound state and an active GTP-bound state. Rho-GTP complexes stimulate downstream signals within the cell. Rho-GTPase activity with the assistance of GTPase-activating proteins (GAPs, red) promotes the hydrolysis of GTP to GDP, resulting in Rho-GDP complexes, which are inactive. Guanine dissociation inhibitors (GDIs, orange) maintain GTPases in their inactive GDP bound state, thereby acting as negative regulators like the GAPs. Guanine nucleotide exchange factors (GEFs, blue), on the other hand, catalyse the exchange of GDP for GTP, thereby serving as signal activators.

GTPase signalling support this hypothesis. The association of the p21-activating kinase PAK3 with MRX (Allen et al. 1998) provides an important example. PAKs are confirmed direct downstream targets of the Rho-GTPases Rac1 and Cdc42; the first MRX-associated *PAK3* mutation identified results in the presence of a stable truncated form of the protein that lacks kinase activity but is capable of binding to the upstream Rho-GTPases Cdc42 and Rac1 (Allen et al. 1998). While the molecular pathway by which this truncation mutant causes the phenotype is not yet clear, the authors discussed the possibility of a simultaneous gain and

loss of function. Other genes implicated in cognitive function can also be linked to downstream targets of Rho-GTPase signalling cascades. For example, a direct target of PAK3, LIM-kinase 1 (LIMK1), has been implicated in cognition. Frangiskakis et al. (1996) showed that hemizygosity of *LIMK1* is responsible for the visuospatial and cognitive impairment associated with Williams syndrome. Likewise, WAVE-1, which has been implicated in motor and cognitive development in mice (Soderling et al. 2003), acts as a scaffolding protein that mediates signalling between Rac-GTPase and the downstream actin related protein (ARP) 2/3 complex (Soderling et al. 2002). In Figure 4, these molecules are depicted within the framework of Rho-GTPase-mediated signal transduction.

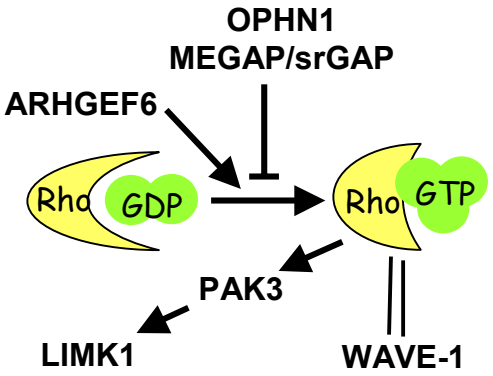


Figure 4: Rho-GTPase signalling and genes with established involvement in cognitive function
OPHN1 and MEGAP/srGAP act as GAPs; ARHGEF6 is a GEF. Downstream effectors of Rho-GTPases include the kinases PAK3 and its target LIMK1; WAVE-1 acts as scaffolding protein that mediates Rho-GTPase signalling.

Rho-GTPases have been linked to many biological processes, and there is a growing bank of information on their multiple functions from numerous *in vitro* and *in vivo* studies (for review, see Bishop and Hall 2000). It is widely accepted that one principle role of Rho-GTPase signalling is in regulation of the actin cytoskeleton (Hall 1998). More specifically, early studies in fibroblasts, which have utilised various constitutively active and dominant negative forms of the three best characterised Rho proteins (RhoA, Rac1, and Cdc42) have highlighted the importance of these molecules for the formation of filopodia, lamellipodia, and stress fibres (Dutartre et al. 1996; Kozma et al. 1997; Manser et al. 1997). Recently, the transfer of this understanding to neurons, verified by studies in both neuron-like cell lines and primary neurons, has highlighted the role of Rho-GTPases in neurite outgrowth and growth cone remodelling (Kozma et al. 1997; Dan et al. 2002), as well as in response to neuronal injury (Dergham et al. 2002; Dubreuil et al. 2003). Numerous reports on related topics

provide somewhat conflicting results; however, there is general agreement that activation of the Rho-GTPases Rac1 and Cdc42 tends to promote neurite outgrowth, whereas activation of RhoA-dependent cascades tend to have inhibitory effects (Nikolic 2002). These studies are discussed in detail, with respect to our findings, in section 4.

1.3 Utilising chromosome abnormalities for identification of candidate MR genes

In the search for MRX genes, molecular characterisation of *de novo* chromosome abnormalities has proved to be a promising starting point. Given the large percentage of non-coding DNA in the human genome, it is not surprising that balanced translocations, which result in no net loss of genetic material and occur at a frequency of approximately 1 in 2000 live births (Warburton 1991), are often present in healthy individuals. However, among carriers of balanced translocations, the frequency of congenital abnormalities is twice that among individuals with normal karyotype (Warburton 1991), suggesting that in approximately half of disease-associated balanced translocations, there is a causal relationship between the translocation and the disorder. Since many balanced translocations do not disrupt genes, it is reasonable to expect that among those that do disrupt genes or control elements, a causal relationship is much more frequent. X-chromosomal genes disrupted by balanced translocations in individuals with developmental anomalies are especially good candidate disease genes, in light of the fact that these genes typically have only one functional copy. This applies in females as it does in males due to fact that female balanced translocation carriers generally exhibit skewed X-inactivation, thereby limiting transcription of X-chromosomal genes to those on the translocated chromosome (Schmidt and Du Sart 1992). Other patients with X-linked mental retardation can be screened for mutations in these candidate genes, in order to verify a causative role in MR. Several MRX genes, including *OPHN1*, *TM4SF2*, and *ARHGEF6* have been identified using exactly this technique (Billuart et al. 1998; Kutsche et al. 2000; Zemni et al. 2000). Given the established heterogeneity of non-syndromic MR, and the absence of specific features that distinguish one genetic form from another, grouping of linkage data from multiple families can be misleading. Further, due to the nature of the disorder, large families for linkage analysis are rare. For these reasons, mapping of disease-associated chromosome rearrangements remains a favourable approach for identifying genes involved in mental retardation. Moreover, our increasing understanding of the molecular pathways involved in cognitive function, combined with the advancement of techniques for studying specific gene function, provides the grounds for a more general search that involves analysis of both X-chromosomal and autosomal genes.

1.4 Aim of the study

To date, more than 30 genes responsible for syndromic forms of XLMR and 14 genes responsible for non-syndromic forms have been identified. Recent studies have shown that most of the MRX genes account for less than 1% of MR in several populations that have been analysed. Given that genes with the highest mutation frequency among XLMR patients are perhaps more readily identified, it is logical to expect that mutations in presently unknown MRX genes may occur at an even lower frequency, and that there are as many as 100 MRX genes that remain to be identified (Ropers et al. 2003). In this study, the aim is to identify candidate genes for MR via molecular analyses on disease-associated balanced translocations. Through functional characterisation of these genes, together with studies in additional patients, we hope to verify their importance in brain development.