Molecular basis of X-linked non-specific mental retardation

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Mental retardation (MR) is a common disorder, affecting 1–3% of the total population. This condition results from failure to develop cognitive abilities and intelligence level appropriate for the age group. Mental retardation is basically a clinically as well as etiologically heterogeneous type of condition and both genetic and non-genetic factors have been found to be involved. There are more than 1000 entries in Online Mendelian Inheritance in Man (OMIM) database under the name of mental retardation. In recent years 15 genes for X linked non-specific mental retardation have been identified which provide important clues regarding molecular and cellular processes involved in signal transduction cascade in central nervous system. Recent advancements in identification and characterization of X-linked non-specific mental retardation genes have been discussed in this review. Understanding of the molecular pathways of disease causing genes would be helpful in developing effective therapeutic approaches for mental retardation.

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Mental retardation (MR) is a common disorder, affecting 1–3% of the total population. It is clinically and etiologically heterogeneous group of conditions, and pathogenesis is largely unknown. Earlier, mental retardation was classified on the basis of IQ test scores as mild, moderate, severe, and profound MR but there were different cutoff levels of IQ scores used by different groups. Moreover, classification of mental retardation on the basis of IQ scores alone may lead to the mislabeling of non-retarded children. In 1992, American association of Mental retardation (AAMR) proposed a new method of categorization which gives emphasis to a person’s capability, taking into account the environments in which the person functions and need for varying levels of support. According to AAMR, mental retardation refers to a fundamental difficulty in learning and the performance of certain daily life skills.

Causes of mental retardation
Approximately, 3% of the total population has an IQ of less than 70 but the cause can be established in less than 50% of all cases. Mild mental retardation (IQ between 50 and 70) is the most frequent (up to 80–85% of all MR) and mostly arises due to low socioeconomic status. There is great variation in the prevalence of MR in different countries e.g. in Sweden and Finland the frequency of MR is reported to be approximately 0.5%. In developing countries like India, malnutrition, in conjunction with socio-cultural deprivation and other health problems related to poverty is probably the most common cause of mild MR. In fact malnutrition and micronutrient deficiency during pregnancy may affect fetal brain development, resulting in mental retardation

Severe and profound MR forms have a lower prevalence, of 0.3 to 0.5%. It has been observed that more severe MR (IQ<50) are generally associated with the other manifestations (may or may not be neurological). The more severe the MR the more likely a precise etiologic diagnosis may be made.

Routine cytogenetic analysis has shown that chromosomal anomalies constitute 40% of severe (IQ<55) and 10–20% of mild mental retardation (IQ 55–70) and among them most common chromosomal cause of mental retardation is trisomy 21 (Down syndrome). It is especially important to identify chromosomal disorders among the non-Mendelian genetic causes of mental retardation, because of high risk of reoccurrence in their families. Mental retardation associated with congenital malformations
and developmental delays are characteristic findings with chromosomal aberrations. Chromosomal subtelomeric rearrangements involving less than 1 or 2 Mb of chromatin are cytogenetically undetectable but could also account for a substantial number of idiopathic MR cases. In fact, such families may provide important clue about the region where candidate gene approaches can be considered.

**X-linked mental retardation (XLMR)**

In 1943, Martin and Bell described a family of X-linked mental retardation without dysmorphic features. Although, this was the first description of sex-linked familial mental retardation, an association between sex and mental retardation was known since long as institutionalized mentally retarded patients showed an excess of males among the severely retarded patients. Reports about many other families with idiopathic mental retardation segregating in X-linked fashion followed the Martin-Bell paper and it was believed that there may be several genes for intellectual function located on the X-chromosome.

X-linked mental retardation accounts for 40% of the total cases of mental retardation among which fragile X-mental retardation is the most common form of inherited mental retardation. The most specific feature of males with XLMR is verbal dysfunction, with lower scores on verbal than on performance IQ testing. Most of the syndromic XLMR and fragile X mental retardation are well characterized.

About 40% of XLMR and 4% of all mental retardation has been attributed to fragile X syndrome. Fragile X-mental retardation, a X-linked semi-dominant condition characterized by CGG repeat expansion at 5' untranslated region of FMR-1 gene, is responsible for the disease in males and 30% of carrier females. Earlier, fragile X-mental retardation was considered as non syndromic mental retardation. Fragile X patients have characteristic clinical and behavioural features hence fragile X syndrome is now included in X-linked syndromic mental retardation. Several others X linked loci influencing intelligence levels are also documented. On the basis genetic origin, XLMR is classified into following main categories:

1. **X-linked recessive and partly dominant disorder (including syndromes, neuromuscular disorders and metabolic disorders)**—This group includes recessively inherited conditions affecting metabolism and mental retardation associated with other neuromuscular manifestations when neurons other than cortical ones are also defective. About 30% cases of Duchenne muscular dystrophy also have mild to moderate mental retardation. Brain dystrophin is expressed in the neuronal soma and dendrites and seems to modulate synaptic integrity, plasticity and signal integration.

2. **Dominant lethal disorders**—This group includes dominant conditions affecting females where skewed X chromosome inactivation is observed in females of these conditions. Eight genes responsible for X-linked dominant conditions are known and three of these have been cloned. All of these genes are lethal in males except EPMD which causes MR and epilepsy in females but males are not affected. It has been hypothesized that homologue cells that inactivate the normal allele die preferentially during embryogenesis or divide less than those with the normal allele on the active X chromosome. In most of the cases, there is random X inactivation, probably because no selection occurs during early stages of development, and female carriers may have different degrees of involvement depending on the fraction of mutant allele on the active X chromosome.

3. **Non-specific XLMR (MRX)**—Non-specific mental retardation (MRX) (i.e. conditions where mental retardation appears to be the only significant clinical finding, without any clear underlying causative factor) is a very common disorder which affects ~1 in 600 males. MRX is clinically homogenous but genetically heterogeneous. Due to absence of any definitive physical features in patients with idiopathic mental retardation, diagnosis of such cases is limited to routine karyotyping.

List of XLMR genes is growing rapidly. At present a total of 203 conditions (137 MRXS and 66 MRX) have been included in update22 (See Table 1). The genes of 117 of these (58 MRXS and 59 MRX) have been regionally mapped and 41 (30 MRXS and 11 MRX) cloned. The recent advances in molecular genetics and positional cloning approaches have made it possible to look for the molecular defects in non-specific MR. Position of some important genes on X chromosome has been shown in Fig 1. Identification of genes have given some rare insights into basic mechanisms of cognition and functioning of central nervous system. Moreover, mutation in several genes can result in non-syndromic as well as in syndromic form of X-linked mental retardation. Therefore, the present knowledge of structure and function of the cloned genes is described below:
FMR-2 gene

FMR-2 gene is located at Xq28 position, 600 kb distal to the FMR-1 gene \(^{24}\). Molecular characterization revealed that individuals expressing fragile site at Xq28 position (FRAXE) had amplification of a GCC repeat adjacent to a CpG island. This condition of trinucleotide repeat amplification represents a class of dynamic mutations where number of GCC repeats increase in successive generations with decrease in age of onset (Genetic anticipation). Normal individuals show 6-25 GCC repeats while individuals expressing the fragile site have >200 copies and their CpG island is fully methylated. In males the full mutation associated with a variable degree of learning difficulty, whilst females appear unaffected. Sequences adjacent to the repeat are highly conserved across animal species and showed mRNA transcripts on Northern blot hybridization. The FMR-2 gene encodes a protein of 1311 amino acids, which probably functions as transcriptional transactivator\(^ {25}\).

The frequency of FMR-2 mutations may be very low because large screening programs in different candidate populations of mentally retarded boys detected none\(^ {26-28}\) or only a few cases of FRAXE MR\(^ {29-31}\). Based on these studies, the prevalence of FRAXE was estimated to be approximately 1:50000 male\(^ {32}\).

Prevalence of FRAXE mutations in Indian population is underrepresented. To the best of our knowledge, there are only two published reports on molecular screening of FRAXE among subjects with non-specific mental retardation. One such study from New Delhi on 124 male patients with idiopathic mental retardation had shown absence of FMR-2 mutation in these patients and 15 GCC repeats were most frequently observed allele in the study population\(^ {33}\). We have analyzed 149 male patients with unknown cause of mental retardation from north India and no patient was found to be positive for FMR-2 mutation\(^ {34}\). Based on these studies, there is strong need to analyze more number of patients with non-specific mental retardation from other parts of Indian subcontinent to find out exact prevalence of FMR-2 in the region.

Oligophrenin 1 gene

This gene is located at Xq12 and encodes for a Rho GTPase-activating protein (RhoGAP). The OPHN1 gene was found to be interrupted in a patient carrying

<table>
<thead>
<tr>
<th>Location X chromosome</th>
<th>Genes</th>
<th>Possible Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>p 22.2</td>
<td>RSK2</td>
<td>Neuronal signaling cascade</td>
</tr>
<tr>
<td>p 22.1</td>
<td>ARX</td>
<td>Unknown</td>
</tr>
<tr>
<td>p 21.3</td>
<td>RLRAP1</td>
<td>Unknown (synaptic plasticity?)</td>
</tr>
<tr>
<td>p 11.4</td>
<td>TM4SF2</td>
<td>Regulation of actin cytoskeleton dynamics</td>
</tr>
<tr>
<td>p 11.2</td>
<td>FGDY</td>
<td></td>
</tr>
<tr>
<td>q 12</td>
<td>OPHN1</td>
<td>Regulation of actin cytoskeleton dynamics/neuronal morphogenesis</td>
</tr>
<tr>
<td></td>
<td>XNP</td>
<td>Unknown</td>
</tr>
<tr>
<td>q 22.3</td>
<td>PAK3</td>
<td>Regulation of actin cytoskeleton dynamics/neuronal morphogenesis</td>
</tr>
<tr>
<td>q 23</td>
<td>FACL4</td>
<td>Fatty acid metabolism</td>
</tr>
<tr>
<td>q 24</td>
<td>AGTR2</td>
<td>Unknown</td>
</tr>
<tr>
<td>q 28</td>
<td>ARHGEF9</td>
<td>Neuronal signaling Transcriptional factor?</td>
</tr>
<tr>
<td></td>
<td>FMR2</td>
<td>Sympathetic nerve fiber &amp; neuronal morphogenesis</td>
</tr>
<tr>
<td></td>
<td>GD01</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>MECP2</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>SLC6A8</td>
<td>Unknown</td>
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</table>

Fig. 1—X chromosome showing the location and name of the genes responsible for X linked non-specific mental retardation with possible function

Table 1—XLMR Genes

<table>
<thead>
<tr>
<th>Class</th>
<th>Number</th>
<th>Mapped</th>
<th>Closed</th>
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<tbody>
<tr>
<td>Malformation syndrome</td>
<td>79</td>
<td>34</td>
<td>7</td>
</tr>
<tr>
<td>Neuromuscular disorder</td>
<td>37</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Metabolic conditions</td>
<td>12</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Dominant conditions</td>
<td>8</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Total MRXS</td>
<td>136</td>
<td>58</td>
<td>26 (33)</td>
</tr>
<tr>
<td>MRX</td>
<td>74</td>
<td>59</td>
<td>15</td>
</tr>
<tr>
<td>Total entries</td>
<td>210</td>
<td>117</td>
<td>41</td>
</tr>
</tbody>
</table>
a X:12 balanced translocation associated with mild mental retardation. A frame-shift mutation causing premature termination and loss of function was described in family MRX60. OPHN1 gene encodes a protein that is highly expressed in fetal brain and contains a domain typical of Rho GTPase-activating proteins. Loss of function or deletion of this gene possibly disturbs signal transduction pathways involved in cell migration and axon outgrowth during development of the nervous system and cause mental retardation.

GD1α

The GD1α dissociation inhibitor-alpha (GD1α or GDIα) genes, located on Xq28, are a family of highly conserved proteins that play a critical role in the recycling of Rab GTPases for vesicular transport. Expression of α isoforms of GDIα is high in the brain, where it is expressed in all areas early in development in post-mitotic neurons. GD1α (which encodes for the protein α GDI) is located at and has been found to be mutated in two families with MRX. GD1α is involved in the control of cycling between the active and inactive status of Rab proteins. Membrane trafficking leading to neuronal development and function of the synapse is dependent on a specific role for GD1α. A missense mutation (arginine to proline) was proposed to affect the protein conformation and possibly its binding to Rab GTPases. Mutations in this gene lead to functional and developmental alterations in the neurons which may account for the severe learning impairment. The frequency of GDPα gene mutations associated with mental retardation is estimated as ~0.5 to 1%, which is 5-10 times less than the frequency of fragile X syndrome.

PAK gene

PAK proteins have been implicated as critical downstream effectors that link Rho GTPases to cytoskeletal reorganization and to nuclear signaling. The larger family of p21-activating kinase (PAK) genes includes human PAK1, PAK2, PAK3, PAK65 and yeast Sic20. A multiplex pedigree (MRX30) in which the gene was mapped to Xq22 was found to have a point mutation in PAK3 gene. Mutation in PAK3 is proposed to interfere with neuronal connections that underlie human cognitive function.

RSK2 gene

RSK2 gene encodes a growth factor-induced kinase that acts in the MAPK activated signaling cascade. Loss of function mutations in RSK2 is responsible in ~50% of Coffin-Lowry syndrome (CLS). Characteristic facial and digital abnormalities and severe MR has been observed in affected males. A large MRX family (MRX19) was mapped to a 42cM region in Xp22, the same interval of CLS. Two affected individuals in the family were examined, and they did not exhibit any physical anomaly and presented mild MR. Further analysis of RSK2 gene in one of the probands detected C to T transition resulting in a R383W amino acid change. The mutation was shown to segregate with the disease in the family and was absent among 250 chromosomes of normal individuals. The mutation caused a 5-6-fold decrease in kinase activity, which is likely not to affect skeletal development but it appears to affect mental process. Further analysis of fibroblast cell lines of patients with CLS shows that RSK2 is involved in signaling processes that may be important in human learning and memory process.

IL1RAPL gene

It is a transmembrane protein that interacts with extracellular molecules and appears to be involved in intracellular signaling. IL1RAPL was identified in a 350-Mb region defined by deletion in a family. This gene shows weak homology to human and mouse interleukin-1 (IL-1) receptor accessory proteins. This gene was confirmed by reverse transcription polymerase chain reaction (RT-PCR), Northern blot analysis, and cDNA library screening. Later, a loss of function mutation was found in a small-unmapped family.

IL1RAPL gene encodes a protein that shares homology with the human and mouse interleukin-1 (IL-1) receptor accessory proteins (IL1RAP) throughout the length of the protein. The IL-1 signaling mechanism allows interaction with IL1RACP, recruitment of IL-1R activated kinase, and activation of an IL-1 dependent cascade of signaling events. IL1RAPL is expressed at a low level in fetal and adult brain, with the highest expression in some regions including the hippocampus, part of the cortex, and the olfactory bulbs. In the mouse, in situ hybridization studies showed that it is first detectable in brain at day E10.5 and is up-regulated at day E12.5. Level of expression then remains same for the rest of embryonic and adult life. It has been postulated that loss of function of IL1RAPL may have an effect in the IL-1 signaling cascade involved in neurotransmitter
release. However, the ligand and the pathways of signaling events remain to be elucidated.

TM4SF2 gene

TM4SF2 was first identified by positional cloning strategy based on a female patient carrying a X:2 balanced translocation. The gene was deduced from the genomic sequence and was interrupted by the breakpoint12. Only 2 out of 33 X-linked families studied carried mutations in the gene. TM4SF2 is ubiquitously expressed, but high expression was observed in brain starting from the very early stages of brain differentiation, at day 10.5. In adult, it is up regulated in the hippocampus and cortex, including primary olfactory cortex.

TM4SF2 is a member of the tetraspan family of proteins known to participate in molecular complexes including β1-integrins. In peripheral blood cells and in cell lines, and through their interactions with β1-integrins, tetraspans mediate diverse cellular progresses such as activation, adhesion, and migration and may participate in events involving RhGTPase and the actin cytoskeleton. Exact role in the CNS is not known.

ARHGEF6 gene

ARHGEF6 gene is located at Xq26 position. Mutations in ARHGEF6 gene that encodes a Rac1/Cdc42 guanine exchange factor were found in patients with X-linked non-specific mental retardation (MRX). ARHGEF6, OPIN1 (oligophrenin) and PAK3 (p21-activating kinase 3) gene products interact with Rho GTPases45 that plays crucial role in neuronal signaling pathways. The molecular mechanism by which mutations in these genes result in deficiencies in neuronal morphology and/or connectivity remains to be elucidated.

MECP2 Gene

Mutations in the MECP2 gene are known to cause Rett syndrome, a severe neurological disorder characterized by developmental delay and mental retardation. A few cases of Rett’s syndrome have been diagnosed in India but majorities of patients still remain undiagnosed17. A series of mutations in the MECP2 gene, different from those causing Rett syndrome, were identified in isolated cases of non-syndromic mental retardation, including A140V, E137G, R167W, P399L, G406n, R453Q, and 387-467del5254. MECP2 mutations were estimated to be responsible for up to 2% of all XLMR, and systematic screening of all patients was proposed55. However, there are marked differences in the phenotypes arising from the loss of function mutations in males and females56,59. Lethal effects have been observed in males when MECP2 gene function is totally abolished but somatic mosaicism and classical features of Rett syndrome are also reported among males50. Marked heterogeneity in clinical features of patients with MECP2 mutation has been reported51. Knockout mouse models of MECP2 showed similar clinical features of Rett syndrome suggesting similar function of this gene in humans and rodents52,53. Further analysis of animal models may give insights regarding the cognitive impairments caused by the mutations in ubiquitously expressed MECP2 gene.

ARX gene

Aristotle-Related Homeobox (ARX) gene spans 1,686 bp and encodes a protein of 562 amino acids. ARX gene is composed of 5 coding exons and encompasses a genomic region of roughly 12.5 kb54. Northern blot and expressed sequence tag (EST) analyses indicated that ARX is expressed predominantly in fetal and adult brain and skeletal muscle. The mouse and zebra fish ARX orthologs are expressed predominantly in forebrain (cerebral cortex) and floor plate, which suggested that ARX protein, is important for the maintenance of specific neuronal subtypes in the cerebral cortex and axonal guidance in the floor plate.

Mutations in the ARX gene in several families with mental retardation (syndromic and non-specific), various forms of epilepsy, including infantile spasms and myoclonic seizures, and dystonia have been reported55. Two recurrent mutations, present in 7 unrelated families, resulted in expansion of polyalanine tracts of the ARX protein. These probably caused protein aggregation, similar to other polyalanine and polyglutamine disorders. A missense mutation within the ARX homeodomain and a truncation mutation have also been described. It has been postulated that mutation of ARX may be a major contributor to X-linked mental retardation and epilepsy.

At least 5 other genes had been identified in which polyalanine expansions cause human disease: HOXD13 in synpolydactyly, RUNX2 in cleidocranial dysplasia, PABP2 in oculoauricular-mental dysostosis, ZIC2 in holoprosencephaly and HOXA13 in hand-foot-genital syndrome55. All other poly A and poly Q expansion disorders are inherited almost exclusively in an autosomal dominant manner, whereas the 2 ARX expansion mutations segregate as X-linked
recessive. Female carriers are not clinically affected and show a random pattern of X-inactivation in blood leukocytes, suggesting a loss rather than a gain of function. Now, function of the ARX gene remains to be identified and whether similar kinds of phenotypes are present in animal models.

**FACL4 gene**

Fatty acid CoA ligase 4 (FACL4) gene belongs to a family of long-chain fatty acid Acyl-CoA synthetase (ACSs) was found to be mutated in some families with X linked non-specific mental retardation. ACSs, the key intermediate in the synthesis of triglycerides, phospholipids, and cholesterol etc., are involved in several cellular pathways like ion distribution, enzyme regulation, vesicle transport, membrane fusion and gene expression. Missense mutation in FACL4 gene causes conformational change of the fatty acid-binding site within the second luciferase domain thereby reducing its activity. Total loss or truncation of FACL4 protein is observed when splice site mutation occur. Defect in fatty acid metabolism in brain may be harmful for neurons when growth cones are converted into synapses and synaptosomal membranes are formed. Disturbance in fatty acid metabolism may affect phosphoinositol mediated signal transduction cascade. FACL4 isoform is highly expressed in hippocampus neurons at the regions thought to be involved in memory process.

**Signal transduction and mental retardation**

Out of 15 genes identified, 5 (OPHN1, TM4SF2, PAK, ARHGEF6 and GD1α) are part of signal transduction pathway involving small GTPases (Fig. 2). The gene products interact with Rho GTPase, a family of small Ras like GTPase that integrate a large number of extracellular and intracellular signals to regulate downstream cellular processes like neuronal proliferation, trafficking, morphology and mobility. The mutations in these genes interacting at different sites within Rho protein signaling pathway leading to mental retardation suggest that abnormalities of signaling mechanisms may be responsible for significant number of non-specific mental retardation. Therefore, mutation analysis in

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**Diagram showing possible molecular interaction of signaling proteins encoded by genes involved in MRX during neuronal spreading.**

Whenever any cell attaches to the receptors on extracellular matrix, several integrin protein clusters (Integrin-linked kinase (ILK), the adaptor protein Paxillin, β-parvin/ParvB, and ARHGEF6 or PIX) are formed and these molecules in turn activate Rho GTPase Rac1 and/or Cdc42 mediated signaling pathways during cell spreading hence, plays crucial role in the reorganization of actin cytoskeleton.
key genes of signal transduction cascade is likely to be a major activity in coming years to unravel molecular defects in mental retardation.

Genetic counseling in MRX families

Mental retarded patients impose a high burden on the society and their families as caring mentally retarded patients cause great trauma to the family. Since, there are no available established protocols for making definite molecular diagnosis (other than fragile X syndrome), carrier detection, and prenatal diagnosis procedures are not possible at present. Genetic Counseling of such cases is also very difficult, as recurrence of idiopathic mental retardation in families is not well defined. Molecular analysis in gene identified so far is mostly in isolated cases and no obvious hotspots have emerged as yet. Screening of point mutations in large number of genes in patients with idiopathic mental retardation is not practical. Therefore, application of recent advancements in DNA microarray and protein microarray based technologies appear promising for diagnosis as well as finding out new genes involved in mental retardation. As the clinical diagnosis is not possible, especially in young children, there is strong need to develop simple molecular tests for mentally retarded children without any obvious etiology. Such tests followed by genetic counseling of the families will greatly help in reducing the burden on the society.

Conclusions

A lot of work has been done in recent past in order to understand the complex genetics behind the mental retardation. Several genes responsible for idiopathic mental retardation have been mapped and cloned. Many of the recently characterized genes encode for proteins that are involved in signaling pathways and in the interactions of neuronal cells with others or extra cellular matrix. These studies reinforce the general conceptualization of non-specific mental retardation as disorders resulting from dysfunctioning of genes required for processes such as remodelling, establishment, and stabilization of connections between neuronal cells. Such processes are crucial for the development of intellectual and cognitive functions. Since these functions begin to evolve mainly in postnatal stages through contact with various stimuli and environments, the potential therapeutic approaches would be the development of drugs that target cellular signaling pathways shown to be implicated in X-linked non-specific mental retardation (MRX).

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