THE MOLECULAR BASIS OF INTELLECTUAL DISABILITY: NOVEL GENES WITH NATURALLY OCCURRING MUTATIONS CAUSING ALTERED GENE EXPRESSION IN THE BRAIN

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TABLE OF CONTENTS

1. ABSTRACT

This review on the genes implicated in mental retardation, and its X-chromosome linked forms, we presented at the symposium *'The Regulation of Gene Expression in the Brain'* (January 23-26, 2003, Heron Island Australia). The main purpose of the review was to highlight the current knowledge of the spectrum of the genes causing mental retardation, provide an insight in to their function(s), where known, and to speculate about the evolutionary processes which shaped such an unexpected concentration of these genes on the human sex chromosome X. Such genes with naturally occurring mutations provide an invaluable opportunity for identifying the pathways essential for the normal function of the brain. Once identified, cellular and animal models can then be used for experimentation.

2. INTRODUCTION

Understanding the normal function of the most complex human organ, the brain, is a challenge of unmatched fundamental scientific and medical significance. A plethora of approaches have been developed and applied to the study of the human brain, including the identification of genes with naturally occurring mutations causing various forms of mental retardation (MR).

MR or intellectual disability is a collection of many disorders encompassing a wide range of neuronal defects of various severity, with or without additional dysmorphic, metabolic or behavioural features. An estimated 1-3% of the population suffer from MR, which ranks highly on health care expenditure of all developed countries. For at least 50% of the affected individuals the cause is never found. Though, most have a genetic basis, this can not be easily determined (1) using existing knowledge and technology. These cases are 'labelled' as idiopathic MR. Only minimal assistance is available for the affected individuals and their families.

Recent advances in the identification of genes causing MR signal the dawn of a new era in the study of the molecular basis of MR and thus normal human brain function. Notably the genes from the human sex chromosome X have been implicated in such phenotypes much more frequently (3-5 times) than one would expect based on the X-chromosome gene and content contribution (about 3% of the gene content and about 5% of the DNA content) to the human genome. Whether the observation of a larger proportion of genes causing MR on the sex chromosome X is some sort of a bias or yet unexplained biological phenomenon, is still debatable. The main purpose of this review, which was presented at the Heron Island Symposium on *'The Regulation of Gene Expression in the Brain'* (January 23-26, 2003, Heron Island Australia) is to bring the current knowledge about the emerging MR gene spectrum and the variety of known and putative new pathways leading to brain malfunction (and/or damage) to the attention of life scientists from disciplines other than human molecular genetics. From the range of the MR disorders and genes only those with a particular type of MR, X-chromosome linked non-syndromic MR (see below for definition), will be discussed.

3. X-LINKED MENTAL RETARDATION, DEFINITION AND INCIDENCE

X-linked mental retardation (XLMR) is simply defined by the presence of MR (IQ<70) and the fact that the causative gene has been mapped to the chromosome X. It has been estimated that 20 to 25% of MR is due to XLMR (2,3), which is about 6-8 times more when compared to the ~3% gene content contribution of the

Table 1. Identified MRX and candidate MRX genes

Gene Name	Gene Function	Year	Reference
MRX Genes			
FMR2	transcriptional co-activator	1996	18
OPHN	Rho-GTPase activating protein	1998	45
PAK3	p21 CDKN1A-activated kinase 3	1998	46
GDI1	GDP dissociation inhibitor 1	1998	47
IIL1RAPL1	interleukin 1 receptor	1999	48
ARHGEF6	Rac/Cdc42 guanine nucleotide exchange factor GEF 6	2000	36
TM4SF2	transmembrane 4 superfamily member 2	2000	49
SLC6A8	solute carrier family 6 neurotrasmitter transporter, creatine member 8	2002	50
FACL4	fatty-acid-Coenzyme A ligase, long-chain 4	2002	51
AGTR2	angiotensin II receptor, type 2	2002	52
MRX genes implicated in syndromic and non-syndromic XLMR			
RPS6KA3	ribosomal protein S6 kinase, 90kDa, polypeptide 3	1999	37
MECP2	methyl-CpG binding protein 2	1999	53, 54
ATRX	chromatin remodeling proteins SWI/SNF	2000	6, 55
FGD1	Rho/Rac guanine nucleotide exchange factor	2002	56
ARX	Aristaless-related homeobox	2002	8, 10, 20
Candidate MRX genes [1]			
ZNF2613	zinc finger protein	1996	57
GRIA3	glutamate receptor, ionotrophic, AMPA 3	1999	58
RPS6KA6	ribosomal protein S6 kinase, 90kDa, polypeptide 6	1999	59
ELK1	member of ETS oncogene family	2000	60
VCX	variable charge, X chromosome	2000	61
NXF5	nuclear RNA export factor 5	2001	62
KLF8	Kruppel-like factor 8	2002	63

[1] Genes isolated from X-chromosome breakpoints and deletions not yet found mutated in familial cases, or found with nucleotide changes not yet shown to be of functional importance.

human X chromosome to the human genome (1 000 human X chromosome genes versus 30-40 000 human genome gene content). Various explanations for this X-chromosome bias are discussed in more detail in the section 7 bellow.

Because of the difficulty of sorting the different forms of XLMR (and MR in general) Mulley *et al.* (4) suggested a basic split of XLMR in two groups, syndromic (MRXS) and non-syndromic (MRX). Families and individuals with syndromic XLMR (like fragile X syndrome, or Hunter syndrome) can be classified as specific clinical entities based on typical physical, metabolic and/or behavioural characteristics (in addition to MR). Families with non-syndromic MR are inseparable and need to be dealt with as a clinically homogeneous group when delineated by detection of mutation in one of the few MRX genes so far described. Non-syndromic XLMR (NSXLMR), even though implying absence of 'syndromic' features, means that such features (if present) may be cryptic or are not consistently found in the affected family members. Thus they can not be used for differential diagnosis. There are now many recorded MRX families where there are no other features present in the affected individuals (almost exclusively boys) than MR. These 'pure cognitive phenotypes' can be very mild to borderline (IQ=70-80), or even absent is some males (eg. FMR2 MR; 5). With the discovery of new X-linked MR genes the delineation between the syndromic and non-syndromic forms of XLMR becomes blurred at the edges as more genes are being identified with mutations in a wide range of

MR phenotypes, syndromic as well as non-syndromic. Good examples of such pleiotropic genes are: *ATRX* (6), *MECP2* (7) and the recently identified gene *ARX* (8-10). The most recent addition to the list could be neuroligin 3 (NLGN3) and perhaps neurologin 4 (NLGN4), which were found to have mutations in males with autism (NLGN3 and NLGN4) and mental retardation (NLGN3) (11).

The incidence of NSXLMR is approximately 1 in 600 (12, 13). Interestingly while there are at least 81 published families (14, 15; and numerous unpublished families) with NSXLMR mapped to human X-chromosome (assigned MRX numbers, ie. MRX 1, MRX2, etc.), there is perhaps only one small family with such gene identified from the autosome (16).

4. MRX GENE COUNT

The first MRX family localisation (MRX1) was published in 1988 (17) and the gene remains elusive. It took a further 8 years untill the first MRX gene (*FMR2*) was identified in 1996 (18, 19). Since then the worldwide resource of large MRX families with associated gene localisations has risen to at least 81 (with many more unpublished). The list of identified genes now stands at 15 (see Table 1). However, only a fraction (~12%) of the families with published localisations, have mutations so far detected in one of the 15 identified MRX genes. This leaves a large proportion of genes for NSXLMR still unaccounted for. Whether we are yet to identify a new major XLMR gene that will account for a significant portion

of these or whether we face the enormous task of identifying many more different XLMR genes remains to be seen.

The recent identification of the *ARX* gene (8-10, 20) shows that XLMR genes affecting many families are likely to be still at large. Using the most recent data on MRX gene identification and a large set of published and unpublished families Ropers *et al.* (15) predicted the existence of such a major XLMR/MRX gene in Xp11.2-p21.

Over the past decade, several attempts to predict the expected number of the X-chromosome genes implicated in MR, and the MRX genes in particular, have been made. These ranged from a minimal estimate of 8 genes based on former gene localisation data (21) to estimates from more recent gene localisation data of at least 22 (22), or up to 50 (3). When including the genes for syndromic XLMR, the number of currently known X-chromosome genes implicated in various forms of MR is over 40 (http://xlmr.interfree.it/home.htm; 3), with additional, at this stage putative MR genes isolated from chromosomal breakpoints but not yet found mutated in familial cases (see Table 1). It is highly likely that the final count of X-chromosome genes, which when mutated cause MR, will exceed 100. Providing that the total gene count on the human X-chromosome is in the range of around 1 000 genes (see http://www.ensembl.org/Homo_sapiens/mapview?chr=X), this would be a significant proportion (~10%) of the chromosome content. Such a contribution to the underlying genetic heterogeneity of mental retardation, and thus cognitive abilities of the human brain from a single chromosome does come as an unexpected surprise.

If we dare to extrapolate such a high level of genetic heterogeneity to the autosomal forms of MR (which represent ~75% of MR), we end up with potentially hundreds of genes (or about 1-2% of human gene count), which in some way may be contributing to the normal function of the human brain (just based on the number of the genes implicated in MR). On the other hand, such genetic heterogeneity can be better comprehended by considering the high molecular (~50% genes are expressed in brain; 23) and structural complexity of the brain. Interestingly, other X-chromosome genes are known, which when transcriptionally silent (eg. *FAM11A*; 24), or mutated (eg. *SEDL*; 25) do not cause any (yet) recognisable brain phenotype. Perhaps some of these 'lack of function' phenotypes can be explained by functional redundancy.

5. MRX GENE EXPRESSION

The majority of the MRX have been identified and isolated as part of the positional candidate and/or candidate gene approaches for the XLMR gene identification (see Figure 1). Mostly they represent novel, yet uncharacterised (and perhaps to some extent incomplete, in terms of the promoter characterisation, alternative splicing, spatial and temporal expression patterns, etc.) genes. Nothing or little is known about their function, detailed spatial and temporal brain expression pattern, or interacting proteins, perhaps with the exception of *PAK3* (p21-activated kinase; 26),

GDI1 (27) and *RSK2* (28), which have been studied more extensively before they have been found to be mutated in XLMR. If we summarise the available expression characteristics of the MRX genes there is no obvious pattern or consistency of gene expression emerging. MRX genes are not brain specific genes. Some are expressed ubiquitously (eg. *ARHGEF6*, *GDI1*), others have a more restricted expression patter (eg. *FMR2*), or the expression is little known (eg. *ILRAPL1*).

MRX genes are expressed during development, where such information has been generated. Many MRX genes are expressed in hippocampus, part of the brain implicated in the processes of learning and memory (29). So far the expression of the known MRX genes has been studied only on a gene by gene basis, apart from a single exploration into the simultaneous expression of multiple MRX genes (30). These investigators used two *in vitro* models of activity-dependent gene regulation, kainate-induced seizures and long-term synaptic potentiation (LTP). Assessed by quantitative PCR some of the tested genes (*PAK3*, *IL1RAPL*, *RSK2* and *TM4SF2*) responded to the stimuli by changing their expression levels. Formally these experiments provide the first link between the MRX gene expression and activity dependent plasticity (30).

Another, perhaps not surprising fact is that all MRX genes appear to be highly conserved with orthologs at least among various vertebrate and in many instances also invertebrate species (eg. *FMR2, GDI1*). This conservation is conducive to their study in model organisms. Indeed, for two of the early MRX genes, *FMR2* and *GDI1*, the knockout mouse models have already been generated (31, 32). Although the utility of mouse knockout models to the study of cognitive phenotypes (often mild) is yet to be established, basic mechanisms are highly likely to be common to both species (32).

6. FROM GENE TO FUNCTION: PATHWAYS IMPLICATED IN NSXLMR

Recently there have been some good reviews describing the functional repertoire of the known MRX genes published (33, 34). Their focus was mostly on those MRX genes and protein products implicated in signaling (33) through Rho GTPase pathways (34). Four of the MRX genes appear to be directly interacting with GTPases (Table 1) and as such signaling is a predominant theme of MRX gene function. The focus on Rho GTPase signaling reflects to some extent the sequence of the discovery of the MRX genes. After the first MRX gene of unknown function, *FMR2* (which was discovered in 1996 by the positional cloning approach using deletions near the *FRAXE* fragile site marker), the discovery of *GDI1* (Rab3a GDI), *OPHN1* (Rho GAP), and *PAK3* (CDC42/Rac1 effector) followed two years after, using candidate (*GDI1* and *PAK3*) and positional candidate (*OPHN1*) approaches. Great expectations (35) and considerable effort has been put in to the investigation of various X-chromosome linked genes associated in any way with the Rho GTPases. However, only one other such gene, *αPIX* (or *ARHGEF6*)

has been discovered two years later with mutation in one single MRX family (36), while additional MRX genes with novel, known or unknown functions, were discovered by positional cloning (*IL1RAPL1*, *TM4SF*2) and candidate gene approaches (*FACL4*, *ARX*).

Moreover, previously identified genes for syndromic X-linked MR were postulated (37) and subsequently found with mutations also in non-syndromic MR cases (eg. *RSK2*, *MECP2*, *ATRX* and *FGDI*). As such the second theme of MRX gene function in global gene silencing (MECP2 and *ATRX*) and direct transcription regulation (FMR2, *ARX*) appeared. However, there was still room for novel, perhaps unexpected functions of additional new MRX genes discovered including the *SLC6A8*, which is a creatine transporter; *FACL4*, a CoA-ligase involved in fatty acid metabolism; and AGTR2, which is a receptor of the hormone angiotensin II. The function of the remaining two known MRX genes, *TM4SF2* and *IL1RAPL1*, coding for transmembrane proteins, is not known.

For those who are hunting for novel MRX genes knowledge of the emerging MRX gene spectrum is very important when prioritising for candidate genes. However, past experience shows that such information is of little help when attempting to simplify the task of finding the next MRX gene. There is still a large number of genes and functions unaccounted for on the human X-chromosome given that for only a small proportion of the MRX families (>81 published) the causative gene has been identified (~12%).

7. X CHROMOSOME MR GENE BIAS, OR AN EVOLUTIONARY FACT?

When comparing the population distribution of IQ scores among males and females (both shaped as bell curves) there are reproducible differences between males and females (38). Simplistically, the male curve appears wider than the female curve, which is interpreted that there is an excess of males over females at both ends of the IQ score distribution. Large population studies confirm this by finding on average a 30% excess of mentally retarded boys with respect to retarded girls. Often, this discrepancy between the numbers of affected boys and girls has been explained by the larger than average gene density for cognitive function on the human chromosome X. Historically, other reasons were also considered such as the greater size of the male head, effect of hormones, or higher social expectations placed on males.

Zechner *et al.* (39) went further, not only supporting the greater than average gene for cognitive function density on the human X-chromosome, but also proposing that such concentration of genes on the human X chromosome is not coincidental, but an important evolutionary process. By searching the human gene, chromosome and morbid phenotype (Online Mendelian Inheritance In Man database: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) databases this study found that mental retardation is at least 3.1 times more frequently associated with X-chromosomal genes than with autosomes (39).

Coming from the evolutionary biology platform Zechner *et al.* (39) proposed that human X-chromosome genes, because of the peculiarities of X-linked inheritance, have been enriched for novel functions, among other mainly functions which contributed to general cognitive ability (but also reproduction). This hypothesis of acquired novel functions for otherwise highly conserved X-chromosome genes as a consequence of female mate choice preference has been further elaborated (40), however, it still awaits direct experimental support. Indirect support for such hypothesis comes from at least two different recent studies. In the first study, Carruth *et al.* (41) studied differences in the brain of mice as a consequence of the presence of one or two copies of the mouse X chromosome and male/female gonadal phenotype. They used a mouse strain with deletion of the *Sry* (testis determining) gene. In this strain the XY⁻ animals have ovaries and thus are considered females. As for the males, the lack of *Sry* is complemented by an autosomal *Sry* transgene, so the XY⁻*Sry* males are fertile. By mating XY⁻*Sry* males and XX females they produced XX and XY⁻ females and XX*Sry* and XY⁻*Sry* males. By measuring and comparing the phenotypic differences of neurons in culture with sex (XY⁻ females versus XX females and XY⁻*Sry* males versus XX*Sry* males) they concluded that the differences were due to the complement of the sex chromosome X.

The second two studies of Enard *et al.* (42) and Gu and Gu, (43) drew attention to the induced gene expression difference in human brain with respect to the brain of the closest human primate relative, chimpanzee. Even though the human and chimpanzee only differ by ~ 1% in genomic DNA sequence, they differ dramatically among others features, in cognitive abilities. It appears that the expression of genes in the human brain has increased during evolution, while in tissues like liver it remained very much the same. Such an observation fits well with the hypothesis of acquired novel functions (and/or expression patterns) of human (X-chromosome inclusive) genes. Perhaps future expression studies using dedicated X-chromosome gene arrays like that produced already (44) will address (support or dispute) this intriguing hypothesis of the 'special evolution and function' of the human X-chromosome bound genes.

8. SUMMARY

Mental retardation is an important medical problem, where environmental and heritable factors contribute equally. The molecular basis for this cognitive phenotype is poorly understood for the genetic forms, where recent advances have merely scratched the surface. A consistent and reproducible excess of ~30% of retarded males over retarded females supports the hypothesis of more than average contribution of the human X-chromosome to human cognitive ability. Direct and indirect scientific evidence is mounting in support of the notion that such enrichment of the human X-chromosome for genes implicated in cognitive function is a consequence of an evolutionary process, which could have shaped our evolution. Although still controversial and not experimentally supported such an hypothesis is plausible.

Since 1996 more than a dozen novel genes implicated in non-syndromic X-linked mental retardation have been discovered. The majority of these genes are defective in only a few families so far worldwide. As a group, the non-syndromic X-linked mental retardation is 4-6 times frequent than fragile X, the most frequent form of familial mental retardation. In spite of the recent progress in XLMR gene identification, the majority of such genes still await identification. MR gene discovery is complicated by considerable genetic heterogeneity as supported by the emerging number of the X-chromosome linked forms. It appears that a large number of genes, each mutated in a small proportion of families can be expected. Although the understanding of the molecular pathways is advancing with each gene discovery, there does not seem to be a common theme. The unravelling of such a theme or themes remains a challenge for the future, to improve the understanding of the molecular basis for MR and to set the foundations for possible future therapeutic interventions.

9. ACKNOWLEDGMENTS

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