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NEWS AND COMMENTARY

Monogenic X-linked mental retardation: Is it as frequent as currently estimated? The paradox of the ARX (Aristaless X) mutations

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Mental retardation affects 30 to 50% more males than females, and X-linked mental retardation (XLMR) is thought to account for the major part of this sex bias. Nonsyndromic XLMR is very heterogeneous, with more than 15 genes identified to date, each of them accounting for a very small proportion of nonsyndromic families. The Aristaless X (ARX) gene is an exception since it was found mutated in 11 of 136 such families, with a highly recurrent mutation (dup24) leading to an expansion of a polyalanine tract in the protein. The rather high frequency of dup24 reported in families with clear X-linked MR (6.6%) contrasts with the very low prevalence of this mutation observed in sporadic male MR (0.13%). We conclude that monogenic XLMR has much lower prevalence in male MR (<10%) than the 23% that would be required to account for a 30% male excess of mental retardation.
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Etiological diagnosis and genetic counselling for mental retardation (MR) is one of the most difficult challenges faced today by clinical geneticists. MR is the most frequent cause of severe handicap in children, with incidence estimates of 0.3–0.5% for moderate to severe MR (IQ≤50) and variable estimates of 1–3% when mild MR (IQ ranging from 50 to 70) is included (reviewed in Stevenson *et al*[1]). Causes of mental retardation can be environmental (perinatal brain ischemia, fetal alcohol syndrome, pre-or postnatal infections), chromosomal (aneuploidies, microdeletion syndromes), monogenic (one finds 1177 mendelian traits or genes in OMIM when searching for mental retardation), but a precise cause is found only in about 50% of cases with moderate to severe MR, and in an even lower proportion for individuals with mild MR. For those cases where no clear etiology can be proposed, one may invoke sporadic occurrence of an unknown single gene defect, multifactorial inheritance, or culturo-familial mental retardation.[1] The latter term reflects the fact that children born to parents with mild or borderline cognitive impairment will also often share a deficient social and cultural environment, which may affect their own intellectual development. Epidemiologic studies in schools and institutions caring for mentally handicapped individuals have repeatedly shown a sex bias, with a 30–50% excess of males over females (reviewed in Stevenson *et al*[1]). Although social biases were first suggested to account for this trend, the description of families with clear X-linked mental retardation, and notably the identification of the fragile X syndrome as a frequent monogenic disease, as well as data from two epidemiological studies,[2,3] led to the current assumption that much of the excess of male MR may be due to X-linked genes.[4–6] A division was made between syndromic X-linked mental retardation (XLMR) characterized by associated clinical, radiologic or biological features that cosegregate with MR in affected families, and nonsyndromic (or 'nonspecific') XLMR. Two old epidemiological studies, carried out in Canada and Australia, are

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the only ones that provide estimates of the incidence of XLMR, based on the systematic identification of sib pairs affected by MR and analysis of their sex distribution. Fishburn *et al*[3] analyzed nonsyndromic moderate MR (that included fragile X families) and reported an incidence of one in 1800 males. In this study, 12 of 45 affected male sibpairs were diagnosed with fragile X, which would correspond to an incidence of fragile X of about one in 6000 in males, which fits rather well with current estimates of one in four to 6000 males in various populations.[7] The study by Herbst and Miller[2] included mild MR and had a somewhat different design, and concluded that incidence of nonsyndromic XLMR may be as high as one in 550. It should be noted that one study failed to find a significant impact of X-linked genes in 'mild idiopathic MR', apart from fragile X.[8]

If monogenic XLMR was to account for an excess of 30% of mentally retarded males over females, and forgetting the case where XLMR can lead to MR, albeit generally milder, in females (as in fragile X syndrome), or even leads to MR almost exclusively in females, such as for Rett syndrome, one would expect that 23% of MR in males is caused by XLMR. As discussed by Stevenson *et al*,[1] several clinical studies reported that XLMR accounts only for 5–8% of male MR, but it is possible that nonsyndromic XLMR was underestimated in such studies, since it cannot be diagnosed in sporadic cases.

The search for genes mutated in syndromic or nonsyndromic forms of XLMR has been very active in recent years. In clearly syndromic forms, one can pool linkage results from different families to lead to an accurate localization facilitating gene identification (as was accomplished for ATRX, Coffin-Lowry or Opitz BBB syndromes). The challenge is much greater for nonsyndromic XLMR as linkage analysis of 60 large families showed extensive genetic heterogeneity, and at least 10 nonoverlapping linkage regions could be defined.[9] Several research groups tackled this difficulty by a combination of approaches: (a) identification of candidate genes located at the breakpoint of X-autosome translocations associated with MR in females, or within X-chromosome deletions in male patients with contiguous gene syndromes that include cognitive deficits, and mutation testing of such candidates in families with established or possible XLMR (the latter being small families with two affected male sibs, or with an affected maternal uncle and nephew); (b) systematic testing of genes in the appropriate candidate region in families informative for linkage analysis (lod score ≥ 2); such families are called MRX families, and the reported ones are consecutively numbered (up to MRX81 in the January update available at www.ggc.org/xlmr.htm). Extremely important for these approaches was the pooling and sharing of a large number of families with established or possible XLMR in the European XLMR Consortium[6,10] or in other collaborations.[11]

These approaches led to exciting success in terms of identification of about 15 genes associated with nonsyndromic XLMR, that surprisingly included a few genes more often associated with syndromic forms like MECP2 (Rett syndrome),[12–14] RSK2 (Coffin–Lowry syndrome),[15] ATRX,[16] or FGD1 (Aarskog syndrome)[17] (see also www.ggc.org/xlmr.htm for further examples). In the latter cases, mutations with milder functional consequences were found in the nonsyndromic families (some missense mutations, or truncating mutations located towards the end of the coding region). In fact, as discussed below for several other XLMR genes (ARX, OPHN1, PQBP1), the boundaries between syndromic and nonsyndromic forms are vanishing.[18]

In terms of genetic counselling and etiological diagnosis, these results were however rather depressing. Almost all of the novel genes were found mutated in only one or two of the 80 large MRX families, and accounted for only a minority of families in which the segregating MR locus was mapped in the corresponding region of the X chromosome. This indicated even more extensive heterogeneity than expected initially, with estimates ranging from 30 to 50 MRX genes to even more than 100 genes.[4–6,19] Based on the comparison of the incidence of the fragile X syndrome in the general population (one in 4–6000 males) and in cohorts of mentally retarded male patients (about 2–2.5%),[20] we estimated that the probability of finding a mutation in a given nonsyndromic XLMR gene, in such patients, would be about one in 500 or less (as a typical X-linked disease has an incidence 10–20 times lower than that of fragile X).[5] It is obvious that, with the present technologies, it is economically not feasible to screen for mutations in these genes in sporadic MR cases.

Initial hopes that the MECP2 gene or the AGTR2 (angiotensin receptor 2) gene may account for a somewhat larger proportion of MR cases were not substantiated. While putative mutations (missense changes) had been found by Couvert *et al*[13] in 2% of MR patients, later studies showed that such changes were in most cases rare variants of no pathological significance (or at most associated with incomplete penetrance of MR), as they were also found in normal relatives.[21] The impact of mutations in AGTR2, initially found in 1.4% of MR patients,[22] also appears controversial.[23,24]

The discovery of mutations of the homeobox gene Aristaless related X (ARX) in Xp22, in a rather large number of families with nonsyndromic or syndromic forms of XLMR, led to a new hope that here at last was a numerically more important gene that would be easily testable, as a highly recurrent mutation was found in a majority of these families. The ARX gene was identified independently by two groups. Stromme *et al*[25] had mapped a syndromic XLMR trait, X-linked infantile spasms syndrome (ISSX, a subgroup of West syndrome) to a 7-megabases region in Xp22, and tested the ARX gene as a

positional candidate expressed in brain. They found mutations in four of five ISSX families, and went on to test six families with other forms of syndromic or nonsyndromic XLMR, in which linkage analysis had mapped the MR locus in the same region. Indeed, they found mutations in two families with Partington's syndrome (MR and dystonic movements of the hands), in two families with MR associated to epilepsy, but also in one of two tested families with apparently nonsyndromic XLMR. Of particular importance was the fact that seven of the nine mutations thus identified corresponded to expansions or duplications of one of two polyalanine tracts present in the Aristaless-related protein. The most frequent mutation, a duplication of 24 bp (called here dup24) resulting in elongation of a polyalanine tract (tract B) from 12 to 20 ala, was found in families with various initial categorizations of phenotypes (one ISSX, two Partington's, one MR with seizures, and one 'nonsyndromic' MRX). In fact, further clinical reanalysis of these families revealed also intrafamilial heterogeneity, with hand dystonia present in 16 of 31 patients with dup24, and infantile spasms or generalized tonic–clonic seizures present each in four patients, four patients being also diagnosed with autism, while only five had 'isolated MR'.[26] The dup24 mutation was found in general to be associated with mild to moderate MR (28 of 31 cases). Another mutation adding 7 ala residues in another polyalanine tract (tract A) was found in two ISSX families.

Bienvenu *et al*[27] tested the ARX gene in nine large nonsyndromic MRX families in which the MR locus had been mapped to Xp22. Their catch was impressive, as they found the dup24 mutation in five of these families, and two missense mutations affecting conserved amino-acid residues in two additional families. In an unpublished work, Schwartz and colleagues in the US identified dup24 mutations in four other 'HUGO numbered' MRX families (MRX 29, 32, 33 and 38, personal communication). Taking all data together, the ARX gene appeared to be a prominently mutated gene in both syndromic and nonsyndromic XLMR families linked to Xp22. Furthermore, as Xp22 is one of the three X chromosome regions in which MRX families map preferentially,[6] ARX mutations account for a larger share of such families than any of the previously identified genes (the fragile X-related genes excluded): they were found in eight of the 80 'official' MRX families, and in three of 56 additional families from the European XLMR consortium (additional data to Ropers *et al*,[6] www.molgen.mpg.de/~abt_rop/NSXLMR/Tabelle-MRX-families.html), that is, in 8.1% of all families, and the dup24 mutation alone accounted for 6.6% of the families (95% CI: 3.5–12.1%). However, these data are derived from analysis of families with clear X-linked segregation of MR. What about the frequency of ARX mutations in sporadic mentally retarded males, or in small families with possible XLMR (two male sibs affected, or a maternal uncle and nephew) (here called pXL families)?.

In the study of Bienvenu *et al*,[27] 148 pXL families were tested and only two mutations were found, a missense change and an insertion of 2 ala in tract A (from 16 to 18 ala). Although these two mutations were not found in 200 control chromosomes, their pathogenic significance was not unambiguously demonstrated (see Jeanpierre[28] or Collins and Schwartz[29]). The same authors found also one dup24 in 40 sporadic MR patients tested. Gronskov *et al*,[30] as reported in the present issue, screened for the ARX polyalanine expansions a large panel of 682 sporadic MR patients. This panel was derived from an initial set of 697 patients, in whom 15 fragile X cases were detected (the 2.15% rate of fragile X mutations is very similar to findings in other MR cohorts, see Biancalana *et al*[20]). In addition, they sequenced the coding region of the gene in 14 families with probable or possible XLMR (11 families with ≥ 3 affected males, three pXL families). Only one dup24 mutation was found, curiously as a clear *de novo* mutation in a single patient from an MRX family (while three other affected males tested negative). In this family, linkage analysis had failed to map a MR locus, which can now be explained by the intrafamilial genetic heterogeneity of MR. Other rare variants were found that corresponded to additions of one or three alanines in the polyalanine tract A. The $+1$ ala was found also in one of 188 controls, and thus appears nonpathologic. One may thus question whether the $+3$ ala, and also the $+2$ ala variant found by Bienvenu *et al*[27] in a pXL family, that were each observed only once in patients but not in controls, are pathogenic or not. Similar negative results have been obtained in yet unpublished series by Schwartz (no dup24 in 577 nonfragile X MR patients, including 132 with MR and seizures, personal communication), and by Frints[31] in Belgium (no dup24 in a cohort of 188 patients that included 57 with MR and infantile spasms). Taken together, only two dup24 were found in 1501 tested MR patients, and none was found in 151 pXL families, indicating that screening for this mutation in sporadic MR cases is very inefficient.

Another disturbing conclusion is the discrepancy between the relatively high rate (6.6%) of dup24 in proven XLMR families (carrying an initial diagnosis of nonsyndromic MR), and the very low rate in sporadic cases, and even in the possible X-linked (pXL) families (Table 1). If indeed 25% of MR in male patients is due to fully penetrant mutations in X-linked genes, one should expect that the yield of dup24 mutations in sporadic cases should be a quarter of that in proven X-linked families, that is, about 1.6% (or at least 1% if one uses a conservative lower estimate of 4% dup24 in true XLMR families), while the observed value is only 0.13% (95% confidence limit is $\leq 0.45\%$). One can estimate also, by Bayesian calculation, that if incidence of XLMR in MR is 25%, a family with two affected sibs would then have a probability of about 69% of being X-linked (this figure depends on recurrence risks for

Table 1 Frequency of the ARX dup24 mutation in MRX families, in potential X-linked MR families and in sporadic 'nonsyndromic' MR cases.

Observed	*n*	*% (95% CI)*	*expected %/n*			
Hypothesis:			*25/6.6*	*25/4*	*10/6.6*	*10/4*
MRX families	9/136 (7/80+2/56)	6.6 (3.5–12.1)				
pXL families	0/151	0 ($\leq$2.2)	4.5/7	2.8/4	2.8/4	1.7/3
sporadic cases	2/1501	0.13 ($\leq$0.45)	1.65/25	1.0/15	0.66/10	0.4/6
pXL+sporadic	2/1652	0.12 ($\leq$0.41)	1.9/32	1.2/19	0.85/14	0.52/9

Hypothesis:% XLMR in MR males/% of dup24 in XLMR. The data on frequency of dup24 in various cohorts are cumulated from Bienvenu *et al*,[27] Gronskov,[30] Frints[31] and Schwartz *et al* (unpublished)(see also www.molgen.mpg.de/~abt_rop/NSXLMR/Tabelle-MRX-families.html). The expected values for occurrence of dup24 in sporadic cases or in families with two affected males (pXL families) were calculated under four different hypotheses that combine a contribution of XLMR to male MR of either 25 or 10%, and a contribution of dup24 to XLMR of either 6.6% (observed value), or of 4%, close to the lower 95% confidence limit. The probability that a pXL family has indeed XLMR was estimated at 69% if XLMR accounts for 25% of male MR, or 43% if XLMR accounts for only 10% of male MR. These estimates are based on bayesian calculations assuming a recurrence risk for non-X-linked MR of 5% (as reviewed by Crow and Tolmie,[32] estimates for recurrence of MR vary widely), and that 1/3 of X-linked cases are due to new mutations.

MR, estimates of which vary quite widely, see Crow and Tolme,[32] and on other unknown variables like mutation rate, size of sibships, and the range is thus about 60–73%). One would have thus expected 7 dup24 in the 151 pXL families tested (or at least four with a more conservative estimate), while none were observed (see Table 1). These pooled data suggest that the proportion of monogenic XLMR in sporadic MR males would be at best 10% (Table 1). Indeed, this would fit with the data on affected male sibpairs of Fishburn *et al*,[3] as the proportion of non-fragile X to fragile X sibpairs was about 3 to 1, and, given the incidence of fragile X mutation (2–2.5% of MR males), this would suggest an incidence of 6–8% for nonfragile X XLMR in nonsyndromic (or at least nonclearly syndromic) patients.

We thus suggest that monogenic MR explains probably less than half of the male excess in MR. Two nonexclusive hypotheses can be proposed to account for the remainder: gender differences in fetal brain development may make the male brain more susceptible to early brain damage,[33] and/or there may be gene polymorphisms on the X that subtly affect cognitive abilities, but without causing overt MR in most of the cases, and thus causing little or no decrease in reproductive fitness. Such alleles would result in MR only when associated with predisposing genetic combinations (of X-linked or autosomal alleles) or environmental conditions. For instance, alleles with a cumulative frequency of 5% and that would give a fourfold relative risk of MR in males would account for a 15% excess of MR in males. Furthermore, both hypotheses would account for the greater male excess observed, at least in some studies, for mild MR than for more severe forms.[34] Affected male sib pair linkage studies might indicate whether some regions of the X chromosome contain preferentially such alleles with a significant effect at the population level. Optimally, one would wish a systematic sequence analysis of the coding regions of all known XLMR genes in such sibpairs, a major undertaking, in order to have a better estimate of the relative contribution of each gene to nonsyndromic MR. One may also search for functional polymorphisms in XLMR genes, or in other X-linked genes that play a role in brain development, and use them in association studies. Although the finding of such putative polymorphisms affecting cognitive function would be of epidemiological and fundamental interest, one would have to be extremely careful to prevent misuse in other contexts.

In any case, the search for new XLMR genes should be pursued actively, together with a careful assessment of their phenotypic associations, as this may help to target mutation screening to selected patient groups. For instance, mutations in the oligophrenin (OPHN1) gene, initially identified as a nonsyndromic XLMR gene, have been recently shown to be associated with cerebellar hypoplasia.[35–37] At present, mutations have been found in 22 of the 80 'official' MRX families. This is, however, likely to be an underestimate of the true impact of known XLMR genes, as not all of these genes have been tested in all families that map in the appropriate X chromosome region, and it is well known that mutation screening of exons, as usually performed, will miss a percentage of mutations (intronic mutations, inversions or duplications…). A recently identified gene that will be interesting to study in cohorts of sporadic cases or of 'pXL' families is PQBP1 (polyglutamine-binding protein 1, a putative transcription regulation factor). Similar to ARX, this gene presents a mutation hot spot (a dinucleotide repeat that is subject to insertions/deletions) and was found mutated in both nonsyndromic MR families, but also in MR associated with microcephaly and/or short stature, including the classic Renpenning syndrome family.[38,39]

More generally, new genetic epidemiology studies on mental retardation should be performed, as improved control of perinatal causes of brain damage may have affected the proportion of genetic *versus* nongenetic cases, and taking into account the recent progress in diagnosis of specific conditions (fragile X, microdeletion syndromes or telomere rearrangements). This would allow a re-evaluation of recurrence risks in function of the sex of proband

(see Crow and Tolmie[32] and Turner and Partington[40]) and give better insight in the proportion of X-linked *versus* other monogenic or polygenic causes of such cognitive impairment.

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