

## **Practical guidelines for molecular testing in non-syndromic mental retardation**

**Disease definition :** Mental retardation (MR) is defined as an intellectual handicap with an intelligence quotient (IQ) of 70 or less with an onset before the age of 18 years. Multiple scales have been used to determine IQ, including the Stanford-Binet scale, Full Scale Intelligence Quotient (FSIQ), and different Wechsler scales. MR is a congenital handicap in contrast to dementia that is a progressive decline of intellectual functions later in life.

**Frequency :** MR affects 1-3 % of the population, but IQs below only account for 0.3-0.5 %.

**Main clinical symptoms :** MR can be associated with (syndromic MR) or without (non-syndromic MR) other features.

**Clinical diagnosis :** When MR is diagnosed, assessment of the IQ by a IQ test is indicated to evaluate the severity and type of MR. In order to provide the best flow chart for molecular testing it is essential to:

- Define the mode of inheritance by pedigree analysis
- Exclude syndromic MR by physical examination to exclude dysmorphic features
- Perform CT scan and/or MRI of the brain to exclude anatomical anomalies
- Perform cytogenetic studies to exclude chromosomal anomalies such as aneuploidies, and CGH array to exclude microdeletions-duplications
- Perform metabolic studies to exclude inborn errors of metabolism (lysosomal enzymes/metabolites, peroxysomal enzymes/metabolites, mitochondrial tests, aminoacids, organic acids)

**Clinical classification :** MR can be classified on clinical grounds as:

- Mild, moderate, severe, or profound
- Syndromic or non-syndromic
- Autosomal recessive, autosomal dominant, sex-linked, or mitochondrial inheritance

MR is considered mild (IQ 50-70), moderate (IQ 35-50), severe (IQ 20-35) and profound (IQ < 20). Sometimes severe MR is referred to all IQs below 50.

More than 1000 disorders are associated with MR including aneuploidies, microdeletions-microduplications, and monogenic defects. Whereas the first two groups nearly always result in syndromic MR, a proportion of the last group is responsible for non-syndromic MR. Some cases are mistakenly classified as non-syndromic MR because the associated symptoms are mild and/or overlooked.

**Inheritance :**

- Mild MR usually is due to a multifactorial interaction between different environmental and genetic factors, although monogenic inheritance occurs in a minority of cases.
- Severe MR usually is caused by a single genetic factor. In about 10 % this is a chromosomal defect that can be visualised by routine cytogenetic studies (eg aneuploidy). In another 10 % a microdeletion or microduplication is present that needs subtelomeric MLPA or CGH array to be demonstrated. Most of the chromosomal defects and microdeletions-microduplications lead to aberrant expression levels of several genes resulting in syndromic MR (contiguous gene syndromes). A significant fraction of severe MR is due to monogenic mutation(s). Monogenic non-syndromic MR can be inherited in an autosomal dominant, autosomal recessive or X-linked manner, whereas mitochondrial inheritance has not been described. Autosomal dominant inheritance is rare, and not a single autosomal dominant locus or gene has been identified. About 10-15 loci and 4 disease genes *PRSS12*, *CRBN5*, *CC2D1A* and *GRIK2* have been implicated in autosomal recessive non-syndromic MR, although many hundreds are expected to be involved.

Although only approximately 10 % of MR is X-linked, there exists an overrepresentation of X-linked loci and genes due to the fact that X-linked pedigrees are more extended than autosomal recessive pedigrees making them more amenable to positional cloning of the disease gene. Currently, more than 30 X-linked genes have been implicated in non-syndromic MR (Table 1).

**Molecular testing** : Non-syndromic MR is very heterogeneous, and more than 100 loci have already been mapped. All loci have been classified as MR (for Mental Retardation), followed by a number indicating the chronological order of identification of the locus. MRA numbers are given to autosomal loci, MRX numbers to X-linked loci. Molecular testing is difficult as more than 30 genes have been shown to be implicated in non-syndromic MR, and none of these genes has a large contribution exceeding 5 %. Furthermore, most mutations are private mutations occurring in a single patient with the exception of the repeat amplifications in *FMR1*, *FMR2* and a common duplication in *ARX*.

- **MRA** : Not a single autosomal dominant locus or gene has been implicated in MR, but 13 loci and 4 disease genes *PRSS12*, *CRBN5*, and *CC2D1A* have been shown to cause autosomal recessive MR. As these genes have only been implicated in a single family, molecular testing is currently not indicated unless in multiplex families where linkage analysis indicates linkage to one of these genes.
- **MRX** : X-linked inheritance can be suspected in families with 2 or more affected males, certainly if present in different generations connected by females that are intellectually normal or have borderline intelligence compatible with heterozygosity for X-linked MR. X-linked MR (MRX) is very heterogeneous with close to 100 loci and more than 30 genes. Many of these genes can also lead to syndromic MR with associate symptoms that might stay unnoticed until careful examination. Currently there are 1è genes considered to give MRX in every patient, whereas mutations in 19 additional genes can result in either MRX or MRXS (Table 1, Figure 1).

Additionally, more than 30 X-linked genes have been implicated in MRXS only, resulting in a total of almost 70 genes on the X chromosome.

Extensive mutation analysis of a large fraction of these genes by the EuroMRX consortium has been able to identify a mutation in 42 % of families with clear X-linked inheritance. Nevertheless, testing of a large number of candidate genes in a single family even when this presents with clear X-linked inheritance is not possible in a diagnostic setting as the cost would be too high. Therefore, diagnostic testing must be based upon specific associated features if present (Table 2). If the MR is non-syndromic in all family members diagnostic testing should be limited to common mutation testing or small genes with a relatively high contribution to MRX such as *ARX*, *MECP2*, and *JARID1C*. However, apart from the repeat size amplifications in *FMR1* and *FMR2* and a duplication in *ARX* no common mutations nor hot spot mutation regions are present in any of these genes. About 10 % of MRX is due the *FMR1*. The other genes most frequently involved are *ARX*, *ATRX*, *FMR1*, *JARID1C*, *L1CAM*, *MECP2*, *PQBP1*, *SLC6A8*, and *SOX3* (Table 1).

An MRX sequencing panel consisting of 8 frequently involved genes, including *ARX*, *PQBP1*, *JARID1C*, *TM4SF2*, *FACL4*, *DLG3*, *FTSJ1*, and *ZNF41*, is available. The estimated detection rate of this panel in non-syndromic XLMR patients is 20-25%.

## **ARX**

Mutations in the *ARX* (Aristaless-related homeobox) gene have been shown to cause nonsyndromic X-linked mental retardation (MRX) as well as syndromic MR such as X-linked lissencephaly with abnormal genitalia (XLAG), Partington syndrome, Proud syndrome, X-linked infantile spasm (West syndrome) and dyskinetic quadriplegia. Mutations can therefore lead to a wide spectrum of anomalies, including seizures, dystonia, spasticity, absent corpus callosum, hydracephaly, lissencephaly, and ambiguous genitalia. The phenotypes without structural malformations, including MRX and X-linked infantile spasms, are associated with missense mutations outside of the homeobox and expansion/deletion of polyA tracts in exon 2. The most common mutation in MRX (also seen in X-linked infantile spasms syndrome, and Partington syndrome) is a

24-bp duplication (c.428\_451 dup) in exon 2 leading to polyA tract expansion. As up to 7 % of MRX is due to mutations in the *ARX* gene, the contribution of this gene to MRX is probably only superseded by the *FMR1* gene. As the *ARX* gene contains only five exons (with 3 of the 4 polyA tracts located in exon 2) diagnostic testing is easy.

### ***ATRX***

Mutations in the *ATRX* gene encoding are responsible for several syndromic forms of MR such as XLMR-hypotonic facies, alpha-thalassemia mental retardation, Carpenter-Waziri syndrome, Holmes-Gang syndrome, Chudley-Lowry syndrome, Juberg-Marsidi syndrome and Smith-Fineman-Myers syndrome. Most patients have syndromic MRX with MR, seizures, early hypotonia and spasticity later, genital, renal and hematological anomalies (anemia, alpha-thalassemia). In some patients *ATRX* duplications are present that are not identified by sequence analysis / DHPLC, suggesting that quantitative analysis to detect copy numbers of the gene may be required in some cases.

### ***FMR1***

Fragile X syndrome is after Down syndrome the most common cause of inherited mental retardation in humans with a population frequency of 1/5000. It represents about 1% of MR and 10 % of MRX. The fragile X syndrome is caused by expansion of a trinucleotide repeat sequence (CGG) in the first exon within the 5' untranslated region of the *FMR1* gene, which causes hypermethylation and consequent silencing of the *FMR1* gene. Only repeat sizes over 200 are associated with MR. Premutations (50-200 repeats) can lead to fragile X-associated Tremor/Ataxia syndrome (FXTAS) in males and premature ovarian failure in females (POF). The full spectrum of the fragile X syndrome also includes behavioral deficits, macroorchidism in postpuberal males, and facial stigmata such as long face, large mandible with prognathism, large anteverted ears. A significant proportion of female carriers of full expansions have symptoms including cognitive and behavioral deficits. More than 99 % of all fragile X patients have repeat amplification, and other *FMR1* mutations are very rare. Consequently, the diagnostic testing can be limited to sizing of the repeat, but

testing still includes Southern blot analysis to visualise full mutations. In view of the low cost of this test and the high prevalence of *FMR1* mutations, this test should be carried out in every male and female with MR.

### ***FMR2***

Fragile E syndrome is also caused by expansion of a trinucleotide repeat sequence (CCG) to more than 200 repeats leading to hypermethylation and downsilencing of the *FMR2* gene. No consistent clinical picture is present, apart from mild intellectual impairment (IQ 50-85), also in a fraction of the female carriers of a full mutation. Severe MR is very rare in fragile E syndrome. Its incidence is estimated to be 1/50-100,000 males, representing only a small fraction of MR, and even MRX. Expansion of the *FMR2* repeat is the only mutation described in the fragile E syndrome. Diagnostic testing is therefore technically easy, and can be combined with *FMR1* repeat analysis in some assays. As most fragile E patients have mild intellectual handicaps they are not institutionalised, and escape most medical attention and genetic testing.

### ***JARID1C***

Mutations in the *JARID1C* gene encoding Jumonji, AT-rich interactive domain 1c protein usually leads to syndromic MR, with most patients having additional features such as behavioral problems, epilepsy, spasticity, microcephaly, short stature, and hypogonadism. The degree of mental retardation in the affected males ranges from mild to severe. Carrier females appeared normal. As *JARID1C* mutations account for an estimate 4 % of MRX, this gene appears to be one of the more frequently mutated genes in MRX/MRXS.

### ***MECP2***

Mutations in the *MECP2* gene encoding methyl-CpG-binding protein not only lead to Rett syndrome in females, but also to diverse phenotypes ranging from severe neonatal encephalopathy, MR associated with spasticity and also non-specific MRX in both males and females. Also PPM-X syndrome (manic-depressive psychosis, pyramidal signs, parkinsonian features, and macroorchidism) and a clinical picture reminiscent of Angelman syndrome can be due to *MECP2* mutations. The severity of MR caused by *MECP2* mutations ranges from

profound to moderate in males and profound to mild in females. As the *MECP2* gene consists of only 3 exons, sequence analysis is technically easy. Microdeletions and microduplications they might not be picked up by standard technologies such as sequencing or DHPLC have been described, and should be excluded by quantitative tests such as MLPA. Microduplications of 0.3-2.3 Mb encompassing the complete *MECP2* gene are characterized by severe MR, neonatal encephalopathy with progressive spasticity and seizures.

### ***L1CAM***

Most patients with mutations in the *L1CAM* gene, encoding the neural cell adhesion molecule L1, have variable features of CRASH syndrome (Corpus callosum hypoplasia, mental Retardation, Adducted thumbs, Spastic paraplegia and Hydrocephalus). This spectrum of L1-associated diseases includes entities such X-linked hydrocephalus, MASA syndrome, X-linked complicated spastic paraplegia type 1 (SPG1) and X-linked agenesis of the corpus callosum. The main clinical features of this spectrum are mental retardation (obligatory), macrocephaly and/or hydrocephalus, corpus callosum hypoplasia, adducted thumbs, and spastic paraplegia. Most carrier females have no symptoms. Only if one of these associated features is present in the patient or one of his maternal male family members the *L1CAM* should be investigated as this gene is large (28 exons). About 70-90% of males with hydrocephalus, at least one of the associated features, and a positive family history, show a mutation in *L1CAM*. Most mutations can be found by sequencing or DHPLC, but gross rearrangements, including large deletions, duplications and large insertions that would not be detected by these methods, have been reported in rare cases.

### ***PQBP1***

Mutations in the *PQBP1* gene encoding polyglutamine-binding protein have been implicated in different forms of syndromic MRXS, including Renpenning syndrome, Sutherland-Haan syndrome, Hamel syndrome, Porteous syndrome and Golabi-Ito-Hall syndrome. MR varies from mild in a minority of patients to severe in most patients. Microcephaly is present in more than 90 % of the

patients, whereas spasticity, small testes and short stature are also frequent findings. Microphthalmia and congenital heart malformations are occasionally found. Therefore, only a minority of patients have non-syndromic MR. Female carriers have normal intelligence, but might have microcephaly. The relative contribution of *PQBP1* mutations to MR is smaller than 1 %, and 4 % to XMR. Certainly if microcephaly is present, the *PQBP1* gene should be analysed. As the gene only counts 4 exons diagnostic testing is relatively easy.

### **SLC6A8**

Mutations in the *SLC6A8* gene encoding member 8 of the solute carrier family 6, a creatine transporter, lead to diminished intracellular levels of creatine. Affected males show mild to severe mental retardation, associated with language impairment, dystonia, ataxia, myopathic facies, seizures, and mood disorders. Few patients show non-syndromic MR. The creatine transporter deficiency leads to increased urine creatine-creatinine ratio. About 1% of males with MR and up to 2 % of MRX patients are estimated to have a *SLC6A8* mutation. A fraction of the carriers exhibit mild mental retardation with behavioral problems. The *SLC6A8* gene is 13 exons long what makes testing feasible although the urine creatine-creatinine ratio can be used as a screening parameter.

### **SOX3**

Mutations of the *SOX3* gene in Xq26-q27 are associated with infundibular a/hypoplasia, callosal abnormalities, anterior hypopituitarism, and ectopic posterior pituitary. The pituitary anomalies are responsible for growth hormone deficiency leading to short stature. Up to 6 % of MRX is thought to be due to *SOX3* mutations, but MRX in contrast to growth hormone deficiency is not an obligate feature of *SOX3* mutations. Both underdosage (inactivation) and over-dosage (micro-duplication) of *SOX3* can lead to this phenotype. Also expansion of a GCC trinucleotide repeat has been described. The duplications should be tested for by MLPA, and the GCC trinucleotide repeat by specific repeat size estimation, as they might not be picked up by standard technologies such as sequencing or DHPLC. As the *SOX3* gene consists of a single but large exon, sequence analysis is technically easy.

- **Unknown inheritance**

The big diagnostic challenge are sporadic cases with MR. In female cases without affected family members only *FMR1* testing might be indicated. In males with MRX diagnostic testing should be limited to common mutation testing (*FMR1* repeat; *FMR2* repeat, *ARX* dup24) in a first diagnostic screen, with reflex testing or small genes with a relatively high contribution to MRX such as *ARX*, *MECP2*, and *JARID1C*. If specific associated features are present in the patient or family members diagnostic testing can be oriented towards specific disease genes (Table 2).

## References

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De Brouwer AP et al. Mutation frequencies of X-linked mental retardation genes in families from the EuroMRX consortium. *Hum Mutat*. 2007; 28: 207-208.

## Databases

[www.ggc.org/xlmr.htm](http://www.ggc.org/xlmr.htm)

[www.euomrx.com](http://www.euomrx.com)

Table 1. Different types of non-syndromic Mental retardation with the proportion of the respective gene, its size, and price indication.

Type	Associated feature	Gene	Protein	Relative gene contribution to MRX (%)	Number of Exons (AA)	Price Indication (Euro)
<b>MRA</b>		CRBN5	Cereblon	0	11 exons (442 AA)	
		CC2D1A	Coiled-coil domain protein	0	23 exons (573 AA)	
		GRIK2	Ionotropic glutamate receptor 6	0	17 exons (869 AA)	
		PRSS12	Neurotrypsin	0	13 exons (875 AA)	
<b>MRX</b>	Epilepsy, autism	AGTR2	Angiotensin receptor 2	small	3 exons (363 AA)	
	Hypotonia	AP1S2	Adaptor protein 1, sigma 2 subunit	small	5 exons (157 AA)	
		ARHGEF6, alpha PIX	Rho guanine nucleotide exchange factor 6	< 1	22 exons (776 AA)	
	<b>Epilepsy, dystonia, lissencephaly, ambiguous genitalia, absent corpus callosum</b>	<b>ARX</b>	<b>Aristaless-related homeobox gene protein</b>	<b>7</b>	<b>5 exons (562 AA)</b>	<b>790</b>

Table 1. Different types of non-syndromic Mental retardation with the proportion of the respective gene, its size, and price indication (continued)

Type	Associated feature	Gene	Protein	Relative gene contribution to MRX	Number of Exons (AA)	Price Indication (Euro)
<b>MRX</b>	<b>Microcephaly, spasticity, genital abnormalities, alpha-thalassemia</b>	<b>ATRX, XNP</b>	<b>Alpha-thalassemia/mental retardation syndrome protein</b>	<b>3</b>	<b>36 exons (2492 AA)</b>	<b>1500</b>
		DLG3	Discs large gene protein		21 exons (849 AA)	1290
	?????????????	EFHC2	EF-hand domain-containing family member C2	< 1	15 exons (749 AA)	
	Aarskog features	FGD1	Faciogenital dysplasia protein		18 exons (961 AA)	1500
		FACL4, ACSL4	Long chain fatty acid-CoA ligase		17 exons (711 AA)	860
	<b>Macroorchidism, long face</b>	<b>FMR1</b>	<b>Fragile site mental retardation 1 protein</b>	<b>10</b>	<b>1 mutation</b>	<b>400</b>
		<b>FMR2</b>	<b>Fragile site mental retardation 2 protein</b>	<b>&lt; 1</b>	<b>1 mutation</b>	<b>170</b>
		FTSJ1	FTSJ1 homolog	2	14 exons (327 AA)	700

Table 1. Different types of non-syndromic Mental retardation with the proportion of the respective gene, its size, and price indication (continued)

Type	Associated feature	Gene	Protein	Relative gene contribution to MRX	Number of Exons (AA)	Price Indication (Euro)
<b>MRX</b>		GDI1	GDP dissociation inhibitor 1	3	11 exons (447 AA)	
	Short stature, facial dysmorphism	GRIA3	Glutamate receptor 3		16 exons (894 AA)	
		IL1RAPL	IL-1 receptor accessory protein	1	10 exons (696 AA)	
	<b>Spasticity, epilepsy, hypogenitalism</b>	<b>JARID1C, SMCX</b>	<b>Jumonji, AT-rich interactive domain 1c protein</b>	<b>4</b>	<b>26 exons (1560 AA)</b>	<b>1450</b>
	<b>Spasticity, Rett features, Angelman features, macroorchidism</b>	<b>MECP2</b>	<b>Methyl-CpG-binding protein 2</b>	<b>6</b>	<b>3 exons (486 AA)</b>	<b>920</b>
		NLGN3	Neuroigin 3		8 exons (848 AA)	980
	??????	NLGN4	Neuroigin 4	3	6 exons (816 AA)	1100
	Ataxia, epilepsy, hypogenitalism, cerebellar hypoplasia, hydrocephaly	OPHN1	Oligophrenin 1	5	25 exons (802 AA)	
		PAK3	P21-activated kinase, Oligophrenin 3		19 exons (559 AA)	
	Cleft lip/palate	PHF8	PHD finger protein 8	1	22 exons (878 AA)	

Table 1. Different types of non-syndromic Mental retardation with the proportion of the respective gene, its size, and price indication (continued)

Type	Associated feature	Gene	Protein	Relative gene contribution to MRX	Number of Exons (AA)	Price Indication (Euro)
<b>MRX</b>	Microcephaly, short stature, small testes	PQBP1	Polyglutamine-binding protein	4	7 exons (265 AA)	500
	Coffin-Lowry features	RPS6KA3	Ribosomal S6 kinase 2 (RSK2)		21 exons (227 AA)	630
	Language deficits, Epilepsy, short stature, spasticity, dystonia, psychiatric symptoms	SLC6A8	Solute carrier family 6, member 8	2	13 exons (635 AA)	1500
	Allan-Herndon-Dudley syndrome, elevated serum T3	SLC16A2, MCT8	Monocarboxylic acid transporter		6 exons (613 AA)	
	Panhypopituitarism, growth hormone deficiency, short stature	SOX3	SRY-Box 3	6	1 exons (446 AA)	
	Rolandic seizures, polymicrogyria	SRPX2	Sushi repeat-containing protein		11 exons (465 AA)	
		TM4SF2	Transmembrane 4 superfamily member 2		8 exons (249 AA)	670

Table 1. Different types of non-syndromic Mental retardation with the proportion of the respective gene, its size, and price indication (continued)

Type	Associated feature	Gene	Protein	Relative gene contribution to MRX	Number of Exons (AA)	Price Indication (Euro)
<b>MRX</b>	FG features, Lujan-Fryns features	UPF3B		?	11 exons (483 AA)	
		ZNF41	Zinc finger 41	1	5 exons (779 AA)	790
		ZNF674	Zinc finger 674	< 1	4 exons (580 AA)	
		ZNF81	Zinc finger 81	1	5 exons (779 AA)	
		ZDHHC15			3 exons (143 AA)	
<b>MRX Panel</b>	Macrocephaly, marfanoid habitus	ARX, PQBP1, JARID1C, TM4SF2, FAFL4, DLG3, FTSJ1, ZNF41	Various		Various	2900

**Table 2. Guidelines for molecular testing in non-syndromic MR.**  
(Symptoms such as hypotonia, seiures are not listed as they are aspecific).

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|--|-----------------|
| • In general                             | FMR1, FMR2, ARX |
| • If macroorchidism                      | FMR1, MECP2     |
| • If infantile spasms                    | ARX             |
| • If hydrocephalus/macrocephaly          | L1CAM           |
| • If microcephaly                        | ATRX, PQBP1     |
| • If microphthalmia                      | PQBP1           |
| • If cerebellar hypoplasia               | OPHN1           |
| • If cleft lip/palate                    | PHF8            |
| • If panhypopituitarism                  | SOX3            |
| • If ambiguous genitalia                 | ARX             |
| • If increased creatine-creatinine ratio | SLC6A8          |
| • If increased serum T3                  | SLC16A2         |
| • If alpha-thalassemia                   | ATRX            |