I. GENETICS

THE OPTIMIZATION OF THE DIAGNOSIS AND MANAGEMENT OF THE PATIENTS AFFECTED BY MENTAL RETARDATION USING MLPA TEST IN THE EVALUATION PROTOCOL

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Abstract

Mental retardation (MR) is a relatively frequent disorder, with heterogeneous etiology and major social implications. Out of the genetic causes, an important part is represented by subtelomeric rearrangements (unidentified by classical analyses). MLPA (multiplex ligation-dependent probe amplification) is a new diagnostic method, cheap and very useful in identifying subtelomeric rearrangements.

Key words: mental retardation, subtelomeric rearrangements, multiplex ligation-dependent probe amplification

Mental retardation (MR) is a very important public health problem, affecting 2-3 % of the population and causing major problems to affected individuals and their families and also to the society. Mental retardation's etiology is various and includes genetic factors (chromosomal abnormalities, monogenic diseases – the most important being X-linked mental retardation and especially Fragile X Syndrome, multifactorial and mitochondrial disorders), environmental factors (infectious diseases, social agents) or simply unknown etiology (nonspecific MR) (Rimoin, 1997; Covic 2004). The proportion of different types of causes is different and in dependence with the degree of MR – so, for moderate and severe MR, genetic agents' contribution is more important than for mild MR, where the environment is having a more important place (especially social agents). In both cases the contribution of unspecific MR is major (de Vries, 2001). Because of that, most of the present's reserches in the field are focused on the study of unspecific MR aiming to identify new genetic factors involved. Recently, it has been observed that subtelomeric rearrangements are having an important contribution (5%) in unspecific MR determinism (Flint, 1995).

Telomeric screening is interesting for 2 reasons :

- Most of the translocations are involving telomeric regions, reason why an investigation of the chromosomal extremities will detect all the abnormalities, no matter the size;
- Adjacent regions of the telomeres are enriched in gene number; rearrangements involving neighbouring DNA is more probably causing phenotypical consequences than other DNA regions (Knight, 2000).

Until recently, screening for the telomeric rearrangements was not possible because of the complexity of the telomeric structure and because of the very expensive investigations. The researches were focused in 2 directions: clinical studies wanted to identify clinical signs that were associated to MR and could suggest the presence of a subtelomeric rearrangement; they also aimed to establish diagnostic scores in order to increase the efficiency of lab investigations (de Vries, 2001; Sandig, 2004); the laboratory work searched for new methods to show better the subtelomeric defect. Out of the clinical studies, we have to mention de Vries' (2001) score projected to increase the efficiency of subtelomeric rearrangements' identification (Table 1).

Table 1: Criteria for patients presenting with submicroscopic rearrangements (de Vries, 2000).

Criteria	Score
Family history of MR	
• Compatible with monogenic inheritance	1
• Uncompatible with monogenic inheritance (including discordant phenotypes)	2
Growth retardation with prenatal onset	2
Postnatal growth abnormalities (for each of them 1 point, with a maximum of 2 points)	
• Microcephaly	1
• Short stature	1
• Macrocephaly	1
• Tall stature	1
2/> facial dysmorphies (especially hipertelorism, nasal or auricular malformations)	2
Extrafacial abnormalities (for each of them 1 point, with a maximum of 2 points); especially:	
• Hand malformations	1
• Cardiac defects	1
• Hipospadias +/- criptorchydism	1

It is recommended that the pacient has a 3/> pts in order to identify an abnormality using MLPA method.

In the laboratory workfield, different techniques were suggested: classical or high resolution cytogenetic techniques (the study of prometaphase chromosomes), different fluorescent in situ hybridization methods – FISH (Knight, 1997; Knight, 2000) and more recently hybridization of the probe and multiplex amplification (MAPH) and the multiplex ligation-dependent probe amplification (MLPA) (Armour, 2002; Sellner, 2004). Standard cytogenetic techniques (a resolution of 400-500 bands) can detect only 5-10 Mb abnormalities, depending on the chromosomal region. High resolution techniques (850-1000 bands) last too long and they are useful only when we look for a specific abnormality in a specific region (it cannot be scanned the entire genome).

FISH techniques are very useful, but the test is very expensive. MAPH is being used for long genes, the method allowing you to study in the same time different parts of the gene (e.g.: the 23 exons of the BRCA1 gene). MLPA is a cheap method (6-10 Euros/test) and very useful for the detection of subtelomeric rearrangements, being considered as an election method in the field (Rooms, 2004). The technique has been recently introduced (2002) and many world specialists took over the technique to appreciate correctly the real frequency of the subtelomeric rearrangement (6,7% - Koolen, 2004), but also to start identifying the clinical picture of every rearrangement (Rossi, 2001).

The main characteristics of the MLPA technique (www.mlpa.com) are:

- It allows the simultaneous testing of 40 different genomic DNA sequences by PCR;
- It can identify sequences differing in a single base- pair;
- The amount of DNA needed is very low (20 μg);
- It needs a termocyclor and an electrophoretic system only;
- The protocol is the same for different applications (detection of subtelomeric rearrangements; detection of aneuploidies - chromosomes 13, 18, 21, X and Y; detection of large deletions or duplications; detection of gene deletions or duplications involved in cancer; detection of deletions / duplications of a single exon in specific genes – BRCA, NF; cuantification of the CpG islands methylation in the promoters of tumours suppression genes);
- Because of the short sequence detection of the probe the method can be used also on the partially degraded DNA (e.g.: DNA extracted from fixed tissues or paraphin blocks);
- The probe is amplified by PCR, not the DNA sample;
- 2 probes are hybridized on the target sequences, then follows probe ligation and amplification of the target;
- Amplification is achieved by multiplex-PCR – all the specific sequences are simultaneously amplified;
- PCR protocol needs only one primer pair for the amplification of all fragments;
- Product's lenght is varying between 130 and 490 bp long, being analysed by electrophoresis.

Subtelomeric rearrangements represent a relatively new described category. The studies in this field are only a few and very simplistic because the methods used until now were very expensive. MLPA seems to be an ideal technique (for now) for the identification of subtelomeric rearrangements, being a cheap method (6-10 E/test) and fit for the identification of submicroscopic chromosomal abnormalities.

Because of the prohibitive price of the earlier methods, but also because of the recently (2002) introduced MLPA method, the number of researches including large patient groups is very limited. Only one study (Koolen, 2004) presented the MLPA results for 210 MR patients.

In the literature, after the identification of an abnormality in a MR child, the parents are tested; if they did not have the abnormality, the defect was considered to be a new mutation that produces the clinical picture of the child. If the abnormality was present also in the normal looking parent, the defect was considered as a polymorphism. If the parents were phenotypically abnormal, presenting the same subtelomeric rearrangement, the defect was considered as a familial one, but there were not many studies testing other members of the family that could be at risk.

There are no articles in the specialised literature to present genetic counselling offered to the family or if the prenatal diagnosis was achieved.

In the literature there are only 2 diagnostic scores (de Vries, 2001; Sandig, 2004), based on a relatively low number of cases.

Considering the small number of identified cases with subtelomeric rearrangements and that the modifications of each telomere is producing a different clinical picture, we can appreciate the early stage in sketching the clinical aspects of MR determined by subtelomeric rearrangements, many clinicians from all over the world still working on this direction.

In Romania subtelomeric rearrangements as a cause of unspecific MR has not yet been studied by any method. The introduction of MLPA in this field would reduce the unidentified causes of mental retardation and increase the efficiency of specific MR diagnosis.

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