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Commentary

Approach to the diagnosis of developmental delay - The changing scenario

Global developmental delay (GDD) refers to the delay in two or more developmental domains (gross motor/ fine motor, cognitive, speech/ language, personal/ social, activities of daily living) in young children less than 5 years of age. Mental retardation (MR) is defined by the American Association on Mental Retardation as "significant limitations both in intellectual functioning and in adaptive behaviour that originates before the age 18 years"[1]. The term mental retardation and/or intellectual disability (ID) usually applies to older children when IQ testing is valid and reliable. It is an aetiologically heterogeneous group of disorders that affects about 2-3 per cent of the general population. Collectively, in up to 50 per cent of the cases, a genetic aetiology has been implicated. Despite extensive evaluation cause for MR remains unknown in about 30-40 per cent of cases which is highly frustrating both for the treating clinician and parents. Identification of aetiology is extremely important not only for managing, prognosticating and allaying parent's anxiety but also for genetic counseling and prenatal diagnosis in subsequent pregnancies. With the rapid evolution of novel molecular techniques and their transfer from bench to bedside the diagnostic evaluation of ID/DD has become more rewarding. Over the past 20 years, continuous advancement in various molecular and cytogenetic techniques has revolutionized the field of clinical genetics by improving the diagnostic yield and unraveling various genetic syndromes.

Genomic imbalances or copy number variations (CNVs) as a group have now increasingly been implicated in the aetiology of mental retardation. These are defined as DNA segments of more than 1 Kb length, which show a variable copy number compared with a reference genome [2]. Such cryptic rearrangements are too small to be detected by conventional cytogenetics. It is also important to emphasize that the results of conventional cytogenetic analysis are highly dependent on operator's skills and the quality of metaphases and the techniques are very labour intensive. Submicroscopic duplications and/or deletions involving the subtelomeric regions are responsible for 5 to 7 per cent of all cases of DD/ID[3]. Different techniques that have been used for the detection of such imbalances are multiprobe fluorescence *in situ* hybridization (FISH) using subtelomeric probes and/ or specific microdeletion probes, different types of FISH techniques, multiplex amplifiable probe hybridization (MAPH), Multiplex ligation-dependent probe amplification (MLPA) and array based comparative genomic hybridization (array CGH) or chromosomal microarray (CMA). Initially, high resolution cytogenetics and FISH studies were considered the gold standard. However, over the years the use of MLPA and CMA has become the choice of diagnostic tests for the evaluation of idiopathic mental retardation. MLPA is especially useful in situations where a clinically well defined microdeletion or microduplication syndrome is suspected by a clinical geneticist, whereas CMA examines the whole genome at a variable resolution for all the cases of idiopathic MR. CMA has resulted in the delineation of various mental retardation syndromes which otherwise would have been unrecognized based solely on the clinical acumen. Although array CGH has supplanted MLPA in the routine diagnostic evaluation of MR, MLPA using various probe sets, still remains an important investigation in the resource poor settings. Both these techniques are not useful in the detection of balanced rearrangements like translocations or inversion as there is no copy number change as such. Cases where an unbalanced change is detected either by MLPA or CMA, a routine cytogenetic analysis with or without FISH probes is useful in determining the origin of rearrangement.

MLPA is a PCR based multiplex technique that studies several genomic regions in a single reaction and allows multiple samples to be handled simultaneously. This method has been reported to be accurate and reliable for identifying deletions and duplications for other genetic disorders such as Duchenne muscular dystrophy[3]. As compared to FISH, it is an efficient, rapid, less labour intensive and cost-effective alternative for the evaluation of unexplained MR. In various studies, based upon the usage of preselection criteria[4], number of patients, the number and types of MLPA kits used, the yield has ranged from 5.3-6.7 per cent[5,6]. CMA analysis is also used for the determination of genomic imbalances but performance is at a much higher resolution. The CMA analysis covers either the target regions that are known to contain clinically significant CNVs or the whole genome depending upon the platform used. The frequency of disease causing CNVs using CMA in patients with unexplained mental retardation with or without dysmorphism has been reported to be as high as 20 per cent[7-9]. Although whole genome array allows the characterization of new genetic syndromes, yet it has a potential for the high number of uncertain results, as benign CNVs are quite prevalent in the phenotypically normal population[10]. Next-generation sequencing (NGS) is a latest emerging technique that allows detection of single-nucleotide changes through the whole genome. Application of this technique has deciphered the aetiology of various mental retardation syndromes *viz.,* Miller syndrome[11], Kabuki syndrome [12] and many others either using whole genome sequencing (WGS) or whole exome sequencing (WES) techniques.

In this issue, Boggula *et al*[13] have assessed the clinical utility of MLPA in the evaluation of the aetiology of unexplained mental retardation, with/without malformations in 203 patients with normal karyotype. MLPA probe sets for subtelomeric regions (P070/ P036) and common microdeletions/ microduplications (P245-A2) were used for all the patients whereas MLPA kit MRX P106 was used in 71% (89/124) male patients with normal MLPA profile using P245-A2 and P070/P036-E1 probe sets. Positive cases with MLPA technique were confirmed using either FISH for 5pter, 4pter, Prader-Willi/Angelman syndrome region and Williams syndrome or follow up confirmatory MLPA probe sets (P372-A1, P373-A1, and P374-A1). The overall detection rate including common microdeletion/ microduplication and subtelomeric probe sets was about 9.3 per cent (19 out of 203). MLPA for X-linked MR was not found to be useful in all the sporadic cases. They have recommended the use of P245-A2 and P070/ P036-E1 probes in all the children with unexplained MR as the most cost-effective and less labour intensive technique. A similar study by Pohovski *et al*[14] has also reported a higher detection rate of about 14 per cent with only MLPA strategy using follow up MLPA kits. In this study, use of follow up MLPA kits has allowed the determination of the approximate size in two thirds of subtelomeric imbalances. In our own experience (unpublished) we have found MLPA to be a useful technique for evaluation of unexplained DD/ID.

With the rapid advancements of DNA based tests and their increasing utility in the clinical practice even for the common health problems, it is important to remember for the physicians that the choice of any genetic test depends upon the accurate clinical diagnosis, specific syndrome, availability of tests, accuracy and reliability of test, diagnostic yield and the cost of the test. There is no single test that covers all the disorders. The aetiological yield can be improved by judicious use of newer diagnostic modalities, and a clinical geneticist evaluation before ordering these tests. It is imperative to have a basic knowledge about these tests, their advantages and the pitfalls in the interpretation. In the developed countries, diagnostic approach to a child with unexplained GDD /ID has been changed and CMA has become a first-line test for evaluation of such patients [7]. However, evidence also shows that a combination of the MLPA kits for subtelomere imbalances and microdeletion syndromes could probably serve as an accurate, reliable, rapid and effective alternative first line test in the evaluation of DD/ID patients where CMA and/or other newer tests like NGS are not available.

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