MLPA Based Detection of Copy Number Variations in Pediatric Patients with Global Developmental Delay/ Intellectual Disability

Original Article

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ABSTRACT

Background: Global developmental delay (GDD)/ Intellectual disability (ID) is characterized by significant limitations in intellectual functioning and adaptive behavior and is frequently associated with developmental delay and various congenital abnormalities. A wide range of environmental factors, in addition to genetic determinants, can affect brain development. Because of the significant clinical and genetic heterogeneity, studying intellectual disability is challenging.
Aim of the study: was to detect chromosomal aberrations and/or copy number variants using various cytogenomics techniques in pediatric patients with intellectual disability.
Our study included 40 selected patients with GDD/ ID with or without multiple congenital anomalies (MCA). Different cytogenomics techniques conducted on our patients revealed normal karyotype in 39 patients and 1 female patient had abnormal karyotype. MLPA subtelomeric screening, and microdeletion/ microduplication screening was done for all patients. Subtelomeric abnormality was detected solely in the patient with abnormal karyotype and chromosomal micro-array was done for this patient only. Screening for microdeletion/ microduplication for all patients revealed 4 positive patients. We concluded that utilizing MLPA technique to screen for subtelomeric regions and microdeletion/ microduplication syndromes, in combination with other cytogenomic methods, is beneficial for diagnosing patients with GDD/ID.

Key Words: Congenital abnormalities, microdeletion, microduplication.

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INTRODUCTION

Global developmental delay (GDD) or intellectual disability (ID) usually associates some genetic disorders as a part of the syndrome, including seizures, autism spectrum disorder and multiple congenital abnormalities (**Shchubelka *et al.*, 2024**). Genetic forms of ID are classified as either monogenic or polygenic, and they contain a wide range of clinical symptoms that are divided into two groups: syndromic ID and non-syndromic ID. Clinical, radiological, or biological symptoms are present in syndromic ID, ranging from well-known syndromes like Angelman syndrome, Rett syndrome, and Fragile X syndrome to less known Kenny-Caffey syndrome, type2 (**Chen *et al.*, 2018**).

ID/MR has two formal definitions that are commonly used. The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) fourth edition definition for ID/ MR and the American Association on Intellectual and Developmental Disabilities (AAIDD) definition for ID are slightly different, but they are essentially the same. Both base the definition on three coexisting features: (1) Significantly subaverage intellectual functioning accompanied by, (2) Deficits or impairments in adaptive skills with, (3) Onset before 18 years of age. Intellectual disability is the new term in DSM-5 replacing the term Mental retardation in DSM-IV. DSM-IV classified the severity based on the cognitive capacity (Intelligence quotient, IQ), but DSM-5 specifies that severity is to be determined by adaptive

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functioning rather than by IQ score. Global developmental delay is diagnosed in "individuals who are unable to undergo systematic assessment of intellectual functioning" including children younger than five years, when clinical severity cannot be reliably assessed during early childhood (**Sharma *et al.*, 2015**).

The most severe cases of ID are caused by genetic factors. Chromosomal rearrangements that are cytogenetically identifiable and submicroscopic chromosomal rearrangements account for about 25% of all cases. X-linked gene abnormalities are responsible for 10–12% of males with ID (**Ropers, 2010**).

A variety of cytogenomic techniques have been used to detect these changes, including the MLPA (multiplex ligation-dependent probe amplification) and array techniques. MLPA is a technique that is used to detect deletions and duplications in genetic diseases of interest, such as the most frequent microdeletion/ microduplication syndromes and subtelomeric regions (**Zanardo *et al.*, 2017**). The application of these techniques led to the identification of a molecular diagnosis in an additional 6-10% (**Willemsen and Kleefstra, 2014**).

Due to its main advantages, Chromosomal microarray (CMA) has aided the detection of novel uncommon DNA copy number variants (CNVs) across the genome. For patients with ID or multiple congenital abnormalities, CMA testing has been recommended as a first-tier cytogenetic diagnostic test (**Miller *et al.*, 2010**).

AIM OF THE STUDY

The study aims at detecting copy number variants using various cytogenomics techniques in pediatric patients with intellectual disability.

MATERIAL AND METHODS

This study was conducted on 40 selected patients who had intellectual disability attending the Clinical Genetics Clinic, Center of Excellence for Human Genetics, National Research Centre because of failure to thrive and global developmental delay.

Inclusion criteria included; 1) intellectual disability (IQ below 70 / global developmental delay) +/- multiple congenital anomalies, 2) both sexes and 3) age under 18 years.

The patient's families were informed about the research protocol and written informed consent was granted from the parents. Patients were subjected to full history taking, past and family history, pedigree analysis, complete physical examination with anthropometric measurements assessment and emphasis on neurological examination.

IQ test was done for patients using (a) Stanford-Binet intelligence scales (**Bain & Allin, 2005**) or Weshsler-Bellevue intelligence scale (**Wechsler D, 1939**).

Conventional cytogenetics analysis was carried out on peripheral blood lymphocytes using GTG banding technique at 550 band level for the patients using standard protocols. DNA extraction by PAXgene kit and measurement of quality and concentration of DNA (PreAnalytix, Hiden, Germany). Multiplex ligation dependent probe amplification (MLPA) assay was done for them using the SALSA MLPA P245 Microdeletion syndrome and P070 Human Telomere probemixes, according to the manufacturer's instruction (MRC-Holland, Netherlands). Fragment Separation by capillary electrophoresis using ABI 3500 (Applied Bio Systems, Foster City, CA, USA). Interpretation of the results was done utilizing Coffalyser.Net™ software (MRC-Holland). Ratios less than 0.75 were considered as deletion, between 0.75 and 1.30 as normal and more than 1.30 as duplication. The collected data was statistically analyzed.

RESULTS

Our study was conducted on 40 selected patients with GDD/ ID with or without multiple congenital anomalies (MCA). Twenty (50%) patients had GDD, 8 patients had mild intellectual disability (20%), 9 patients had moderate intellectual disability (22.5%) and 3 patients with severe intellectual disability (7.5%). Five patients revealed chromosomal structural abnormalities or copy number variants using various cytogenomics technique. Four of them was detected using MLPA and one had abnormal karyotype which was confirmed by chromosomal microarray and previously published. Two of them had GDD and 3 had mild ID.

Parental consanguinity was present in 23 patients representing 57.5%, family history of ID was present in 23 patients representing 57.5% and family history of miscarriage in 8 patients representing 20%. On examination facial dysmorphic features (e.g.; frontal bossing, dolicocephaly, fascial asymmetry, low anterior hairline, long eye lashes, arched eyebrows, synophoros, epicanthal folds, hypertelorism, hypotelorism, depressed nasal bridge, anteverted nostrils, peaked nose, cupped ears, low set ears, long or short philtrum, cleft lip and palate were noted in 38 patients representing 95%. Non-facial dysmorphic features in the form of brachydactyly, arachnodactyly, simian creases, overriding toes and dysplastic nipples were present in 30 patients representing 75%. Other congenital anomalies (e.g. congenital heart disease, brain anomalies, umbilical/ inguinal hernia, and unilateral renal agenesis) were present in 31 patients representing 77.5%. Abnormal growth including low birth weight, failure to thrive and stunted growth was found in 13 patients representing 32.5%, short stature in 11 patients representing (27.5%) and convulsions in 2 patients representing 5%.

Different cytogenomics techniques conducted on our patients revealed normal karyotype in 39 patients, representing 97.5%. Only 1 female patient, representing 2.5%, had abnormal karyotype described as 46,XX,add(18)(q23).

MLPA subtelomeric screening using P070 probemix and microdeletion/ microduplication screening using P245 probemix were done for all patients. Subtelomeric abnormality was detected solely in the patient with abnormal karyotype. Her subtelomeric screening revealed deletion 18q subtelomere. This case was previously published by us (**Mahrous *et al.*, 2021**). No other subtelomeric aberrations were detected in the other 39 (97.5%) patients with normal karyotype.

Screening for microdeletion/ microduplication for all patients revealed 4 positive patients representing 10%. One patient had 5q35.3 deletion causing Sotos syndrome. Two patients had 22q13 deletion causing Phelan-McDermid syndrome. One patient had 15q11.2 deletion causing Prader-Willi syndrome. Thus, the total positive cases by MLPA were 5 patients, representing 12.5%.

The 1st patient was a female, 1 year and 10 months old, presented to the clinic with global developmental delay and large head. There were dysmorphic features including macrocephaly, dolichocephaly, frontal bossing, deep seated eyes, arched eyebrows, high arched palate, low set large ears and short neck. Anthropometric measurements, at time of referral were as follows; weight =11kg (-0.7SD), head circumference =52.5 (+3.3 SD) and height =85 cm (+0.3SD). MLPA analysis using P245 microdeletion probemix revealed 5q35.3 deletion causing Sotos syndrome. (Figure 1)

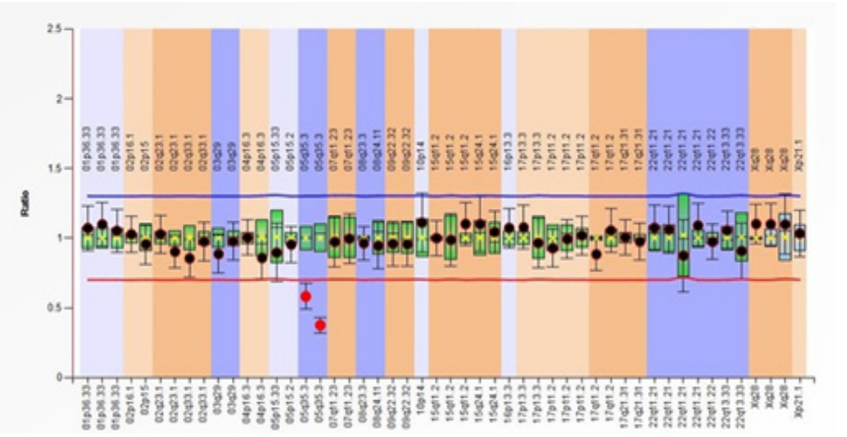

Figure 1: Ratio chart of patient 1 showing 5q35.3 deletion causing Sotos syndrome.

The 2nd patient was a female, 3.6 years old, she presented to our clinic with global developmental delay and autistic features. Her birth weight was average. MLPA analysis using P245 microdeletion probemix revealed 22q13 deletion causing Phelan-Mcdermid syndrome. (Figure 2)

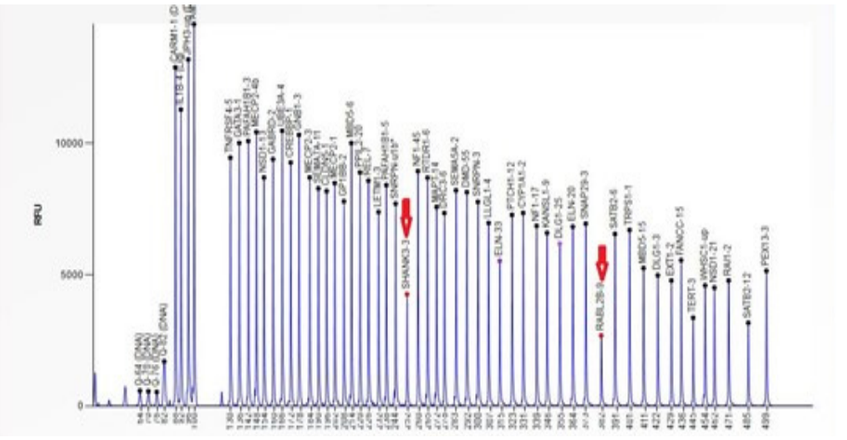

Figure 2: Electropherogram of patient 2 showing 22q13 deletion causing Phelan-Mcdermid syndrome.

The 3rd patient was a female, 6.6 years old, presented to the clinic with delayed mental milestones. MLPA analysis using P245 microdeletion probemix revealed 22q13 deletion causing Phelan-Mcdermid syndrome. (Figure 3)

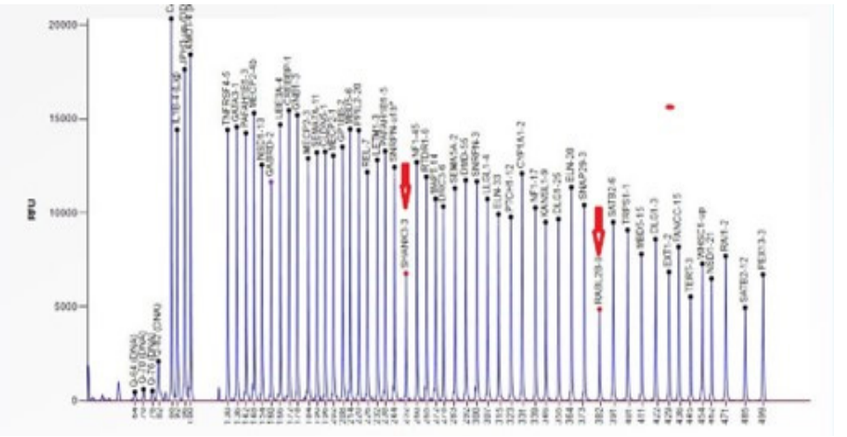

Figure 3: Electropherogram of patient 3 showing 22q13 deletion causing Phelan-Mcdermid syndrome.

The 4th patient was a male, 6 years old, presented to the clinic with mild intellectual disability (IQ=66) and dysmorphic features. MLPA analysis using P245 microdeletion probemix revealed 15q11.2 deletion causing Prader-Willi syndrome. (Figure 4)

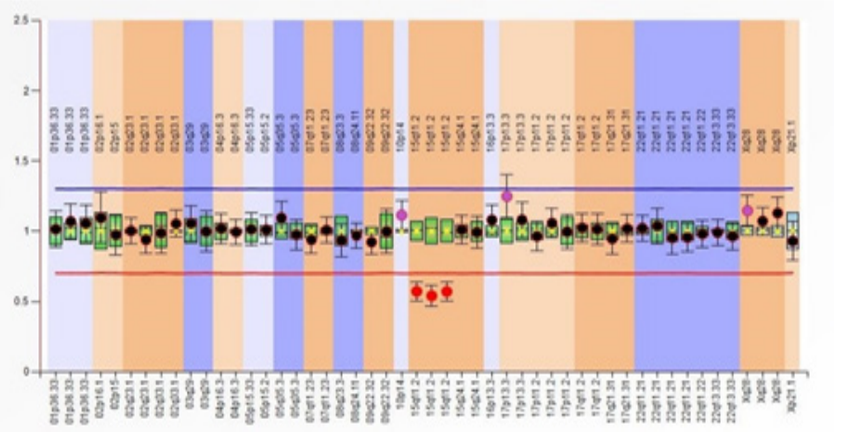

Figure 4: Ratio chart of patient 4 showing 15q11.2 deletion causing Prader-Willi syndrome

DISCUSSION

Multiplex ligation-dependent probe amplification (MLPA) is indeed the method of choice for screening large cohorts of individuals with DD/ID since it is reasonably rapid, easy to interpret, and cost-effective (**Pohovski *et al.*, 2013**).

Compared to **Abdel Azeem *et al.* 2009,** in our study patients with mild ID represent 20 % but, in their study, they represented 67.5%. There was a statistically significant difference in the proportion of infants who developed GDD between the literature and data resulting from this study (p<0. 001). In the literature, there is a wide variation in the prevalence of GDD throughout the world. Shatla and Goweda, 2020 stated that combined developmental delays prevalence is estimated to be 2.7% of children. Another study by Almahmoud and Abushaikha, 2023 stated that GDD affect 2%–3% of the worldwide population. In our study, patients was diagnosed by GDD which considered an increased rate compared to literature. This could be attributed to the use of diverse tools for evaluating development, studies being led in different societies and to our small sample size.

Compared to an Egyptian study by **Abdel Azeem *et al.*, 2009** study which assessed the genetic and epidemiologic aspects of mental retardation and **Elmasry *et al*, 2020** who made a study to identify the prevalence and risk factors of intellectual disabilities in children. In **Abdel Azeem *et al.*, 2009** study 60% of their study group showed positive consanguinity, 15% had congenital anomalies, 12 % had

abnormal growth, 34.7% with family h/o of int. disability, 14.7% suffered from convulsions and 12.6 had short stature. In a study by **Elmasry *et al,* 2020**, 8.89% had dysmorphic features. This can be explained by the small sample size and the sampling technique. Also, in our study most patients were not presented by isolated GDD/ID.

Regarding the different types of genetic testing used to establish an etiological diagnosis in GDD/ID, an extensive review of the literature regarding the evaluation possibilities of the children affected by GDD/ID determined the overall karyotype detection rate to be 3.7%, with Down syndrome, sex chromosome aneuploidies, and unbalanced translocations/deletion syndromes being the most frequently encountered anomalies (**Miclea *et al.*, 2021**). In our study the detection of chromosomal abnormalities by conventional karyotype was 2.5% (1 in 40 patients). Comparing our results to literature it coincides with overall detection rate of karyotype in GDD/ID.

Our result coincides with the study reported by **John *et al.***, (2013); In their study they screened 122 patients with GDD/ID that were selected to study cytogenetic and MLPA analysis after a thorough clinical investigation. All patients had apparently normal karyotypes. SALSA MLPA P245-A2 kit was applied to 122 patients and P 070 and P 036 was applied to 75 patients only. All their patients showed no subtelomeric copy number variance in patients with normal karyotype like our study. It is worth noting that our sole patient who had subtelomeric deletion showed an abnormal karyotype. Eleven out of 122 (9%) submicroscopic deletions were identified in their study by MLPA kit for microdeletion syndromes. Comparing to their results subtelomeric screening in both studies had not detected any copy number variance in patients with normal karyotype. However, the microdeletion/ microduplication syndromes detection rate was approximately the same as in our patients. In their study 3 out of 11 patients (11 positive cases out of 122 total) showed micro-deletion in SHANK3 using P245-A2 kit, which has a clear clinical significance as it overlaps with a known microdeletion syndrome, the Phelan-McDermid syndrome. Similarly, we diagnosed 2 out of 5 (5 positive cases out of 40 total) patients with the same syndrome.

On the other hand, a lower detection rate was reported by **Miclea *et al.***, (2021). They conducted a study on a total of 501 patients with GDD/ID, their patients were analyzed using SALSA MLPA probemix P245 Microdeletion Syndromes-1A. 25 patients (5%) were diagnosed with a microdeletion/ microduplication syndrome. Amongst them, 7 of 25 (30%) with clinical suggestion that confirmed the diagnosis, the clinical findings in the other cases were not suggestive of a distinct syndrome. The most frequent diagnosed syndromes in their study were DiGeorge, Prader–Willi, Angelman, Langer–Giedion syndromes and 17q21.31, 15q24 microdeletions.

Another cohort of 107 children with GDD/ID conducted by **Mohan *et al.*,** (2016) for microdeletion/ microduplication syndrome screening using SALSA MLPA P064 probe kit that was conducted for 100 children, a detection rate of 2% was reported. Microdeletion in the 15q11.2 region causing Prader Willi syndrome was detected in one patient and the other showed a microduplication in the 7q11.23 region causing Williams syndrome. The detection of subtelomeric rearrangements in seven children with known chromosomal abnormalities identified earlier by FISH was compared using MLPA. Two children had microdeletions, while five children had unbalanced translocations that resulted in a microdeletion and a microduplication. The MLPA technique detected microdeletions in all seven children, but the microduplications in two of five children with unbalanced translocations were not detected.

We concluded that the detection rate of MLPA technique for screening of subtelomeres and microdeletion/ microduplication syndromes in literature had varied among different studies with the lowest detection rate 0% for subtelomeric regions reported by **John *et al.*, (2013)** and 2% for microdeletion/ microduplication syndrome reported by **Mohan *et al.,* (2016)**. The most frequent microdeletion/ microduplication syndromes in various studies were DiGeorge, Prader–Willi, Angelman, Langer–Giedion syndromes and 17q21.31, 15q24 microdeletions. In our study the overall detection rate was 12.5%. 10% for microdeletion/ microduplication syndromes, 0% for subtelomeric screening of patients with normal karyotype and 2.5 % for subtelomeric screening in a patient with unbalanced chromosomal aberration by karyotype. A more recent study by **Srivastava *et al.,* 2022** almost had similar detection rate as our study of 13.5% in India conducted on 332 children with DD/ID with or without facial dysmorphism and congenital malformations by MLPA using P245 probe. Our increased detection rate compared to other studies may be explained in our study by the majority of patients had MCA and that submicroscopic imbalances may be responsible for a large portion of chromosomal abnormalities in MCA/MR patients.

We suggest that subtelomeric screening using MLPA subtelomeric kit had a low detection rate overall. These may be explained by the presence of only one probe covering an exact area of the subtelomere not all the entire subtelomeric region thus lessens its sensitivity.

The use of different MLPA probes as microdeletion syndromes probes, methylation kits and X-linked mental retardation kits could raise the copy number variants detection of certain loci and genes to identify the genetic causes of GDD/ID. MLPA analysis is a sensitive and specific technique for screening of GDD/ID before using CMA for relatively low cost compared to costs for microarray.

STATEMENTS

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STATEMENT OF ETHICS

The study conducted according to the guidelines of the Medical Research Ethics Committee of the National Research Centre based on the World Medical Association Declaration of Helsinki, and an informed consent was taken from the patient's guardians.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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AUTHORS' CONTRIBUTIONS

All authors have made substantial contributions to the conception of the work, analysis and interpretation of data, participated in revising the work, responsible for all aspects of the work in ensuring that questions related to the accuracy of the work are appropriately investigated, and they all approved the final version to be published.

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