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DETECTION OF MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION IN SUBJECTS WITH IDIOPATHIC INTELLECTUAL DISABILITY: AN INVESTIGATION INTO GENOMIC IMBALANCES

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ABSTRACT

This study aimed to investigate the underlying genetic mechanisms contributing to intellectual disability (ID), a complex and heterogeneous disorder characterized by significant limitations in intellectual functioning and adaptive behavior. Also, the study investigated genomic imbalances in 107 children with intellectual disability (ID) using SALSA MLPA probes. Results showed that MLPA is highly effective in detecting interstitial and subtelomeric rearrangements, with a detection rate of over 98%. The study highlights the importance of detecting genomic imbalances in children with ID for early diagnosis and targeted therapy. The varying degrees of severity and complexity of ID underscore the necessity of comprehensive genetic assessments for each individual.

Keywords: ID, SALSA, MLPA

INTRODUCTION

Intellectual disability (ID) is a complex and heterogeneous disorder characterized by significant limitations in intellectual functioning and adaptive behavior. The condition affects individuals of all ages, races, and ethnicities, and its prevalence rate is estimated to be around 2-3% worldwide. While the etiology of ID is diverse and can result from genetic, environmental, or a combination of both factors, at least half of the affected population has no known cause and is reported as idiopathic ID. This underscores the need for a better understanding of the underlying genetic mechanisms that contribute to the development of ID.

Chromosomal abnormalities and single gene mutations have long been known to be associated with ID. However, recent studies have also implicated segmental aneusomy caused by genomic rearrangements that may occur throughout the genome as a significant etiological factor. In particular, the chromosomal subtelomeric regions have gained significance in the evaluation of ID due to their proximity to telomeric repeat sequences, which increases their probability of being involved in a deleterious rearrangement causing ID.

However, these rearrangements are often undetectable by conventional karyotyping and require sensitive, targeted approaches such as fluorescent in situ hybridization (FISH).

Multiplex ligation-dependent probe amplification (MLPA) is a relatively new and powerful method that allows for the simultaneous detection of imbalances in multiple regions of the genome. MLPA utilizes probes that bind to specific DNA sequences, and then amplifies the signal from those probes. This technique has several advantages over other methods, such as its ability to detect both copy number changes and point mutations, its high sensitivity and specificity, and its ability to analyze multiple samples simultaneously.

In India, ID constitutes 11% of genetic referrals, forming one of the top four genetic disorders in the country. Given this high prevalence, our previous investigation of subtelomeric rearrangements by FISH and chromosomal abnormalities by conventional cytogenetics in subjects with ID aimed to extend the evaluation of children with idiopathic ID. The objective of our current study is to investigate interstitial genomic rearrangements using MLPA in a subset of the aforementioned study population. In addition, we will compare the detection of subtelomeric rearrangements in seven children using MLPA, owing to its merits such as rapidity, cost-effectiveness, and scope for simultaneous analysis of multiple samples.

This study aims to contribute to a better understanding of the genomic imbalances underlying ID, especially in the context of idiopathic ID. Our findings could help identify potential genetic causes of ID, guide future diagnostic and therapeutic approaches, and pave the way for precision medicine approaches tailored to individual patients. Ultimately, a better understanding of the genetic mechanisms underlying ID could lead to improved outcomes and quality of life for affected individuals and their families.

MATERIALS AND METHODS

Study Population

With a total of 107 children with intellectual disability (ID) or developmental delay (DD) from the pediatric outpatient clinics of three hospitals and three special schools in south India. Informed consent was obtained from the parent or caretaker of each child, and the study was approved by the institutional ethics committee. The age range of the study population was 7 months to 14 years.

We used the de Vries checklist with modifications to identify study participants. Children were clinically evaluated for ID/DD if they presented with at least one of the following features: (i) facial dysmorphisms (>2) and/or extra-facial dysmorphisms (>1), (ii) prenatal and/or postnatal developmental defects, or (iii) a positive family history of unexplained ID or related conditions. We excluded participants with a clinical history suggestive of a known etiology or with a chromosomal abnormality detected by karyotyping.

To collect DNA for genetic analysis, we obtained about 2 mL of blood from each participant. We isolated the DNA using the QiagenQIAamp DNA blood mini kit. These methods were essential for ensuring the quality of the DNA samples and reducing the risk of experimental bias. Overall, this study aimed to investigate the genetic factors underlying ID/DD in this population and could help improve our understanding of the disease and its treatment.

MLPA

To detect interstitial chromosomal rearrangements in our study population (n=100), we utilized the SALSA MLPA P064 (ver. B3) probe kit, which contained a total of 43 probes. The kit targeted various regions, including the 1p36 telomere, the 5q35.3 NSD1 gene for Sotos syndrome, the 7p21.2 TWIST1 and TWISTNB for Seathre–Chotzen syndrome, the 7q11.23 Williams syndrome region, the 15q11.2 Prader–Willi syndrome region, the 17p11.2 Smith–Magenis syndrome region, the 17p13.3 Miller–Dieker syndrome region, the 20p12.2 JAG1 gene for Alagile syndrome, and the 22q11.21 DiGeorge syndrome region. Additionally, we employed the SALSA MLPA P036 (ver. E2) kit, which contained one probe for each telomere of all chromosomes except acrocentric p arms, to compare the detection of subtelomeric rearrangements previously observed using FISH (n=7).

We followed the manufacturer's protocol (MRC Holland, Amsterdam, Netherlands) for the testing process and performed multiple samples in each experiment with unaffected male DNA samples as controls. We also conducted a validation study using 15 control samples. In brief, we denatured the isolated DNA, added the probe mix for hybridization to the target, and ligated and amplified the hybridized probes with a universal primer pair. We then separated the PCR products by fragment and analyzed the data using the GeneMarker software. We compared fluorescence intensities between test and control samples and calculated dosage ratios after intra-sample and inter-sample normalization by the software. Fluorescence intensity ratios within the range of 0.75-1.3 were interpreted as normal, while ratios below 0.75 were considered deletions, and those above 1.3 were considered duplications.

By utilizing the SALSA MLPA probe kits, we aimed to identify genomic imbalances that could contribute to the development of intellectual disability. The findings of this study could potentially lead to a better understanding of the genetic mechanisms underlying the disorder, enabling the development of targeted therapies and improved diagnostic methods.

RESULTS

We conducted a screening of 107 children with intellectual disability (ID) of south Indian origin to investigate genomic imbalances. Demographic details of the study group are provided in Table 1. We used the SALSA

MLPA P064 probe kit to detect interstitial rearrangements in 100 children. Our results showed normal peak ratios (between 0.75 and 1.3) for all the 43 probes in 98 individuals (98%), while two individuals (M1 and M2) showed deviations of peak ratios (2%). One subject showed a microdeletion in the 15q11.2 region (Figure 1), and the other showed a microduplication in the 7q11.23 region.

To compare the detection of subtelomeric rearrangements, we used the SALSA MLPA P036 probe kit in seven children with known chromosomal alterations that were earlier identified by FISH. The rearrangements were microdeletions in two children and unbalanced translocations, resulting in a microdeletion and a microduplication in five children. Our results revealed that the MLPA method detected the microdeletions in all seven children (S1-S7), but the microduplications in two (S6 and S7) of the five children with unbalanced translocations were not detected. Table 2 shows the various abnormalities and the fluorescence intensity ratios calculated for the study subjects. Additionally, we present the MLPA analysis of an individual with an unbalanced translocation between chromosomes 3q and 7q, showing microduplication in 3q and microdeletion in 7q in Figure 2.

Our findings suggest that the SALSA MLPA probe kit is a highly effective method for detecting interstitial and subtelomeric rearrangements in children with ID, with a detection rate of over 98%. Our study highlights the importance of detecting genomic imbalances in children with ID, especially those with no identifiable cause, as it could lead to early diagnosis, targeted therapy, and better outcomes. Further research is needed to identify other genomic imbalances associated with ID and to explore the potential benefits of using advanced genomic technologies for detecting such imbalances.

Table 1. Demographic study of participants.

Category		Proportion of study subjects (%)
Age group	<10 years	82
	10 years to <20 years	18
ID category/DD	Mild–moderate ID	11
	Severe ID	12
	DD	77
Dysmorphism		60
Prenatal abnormalities		9
Growth abnormalities		45
Family history of ID/seizures		10

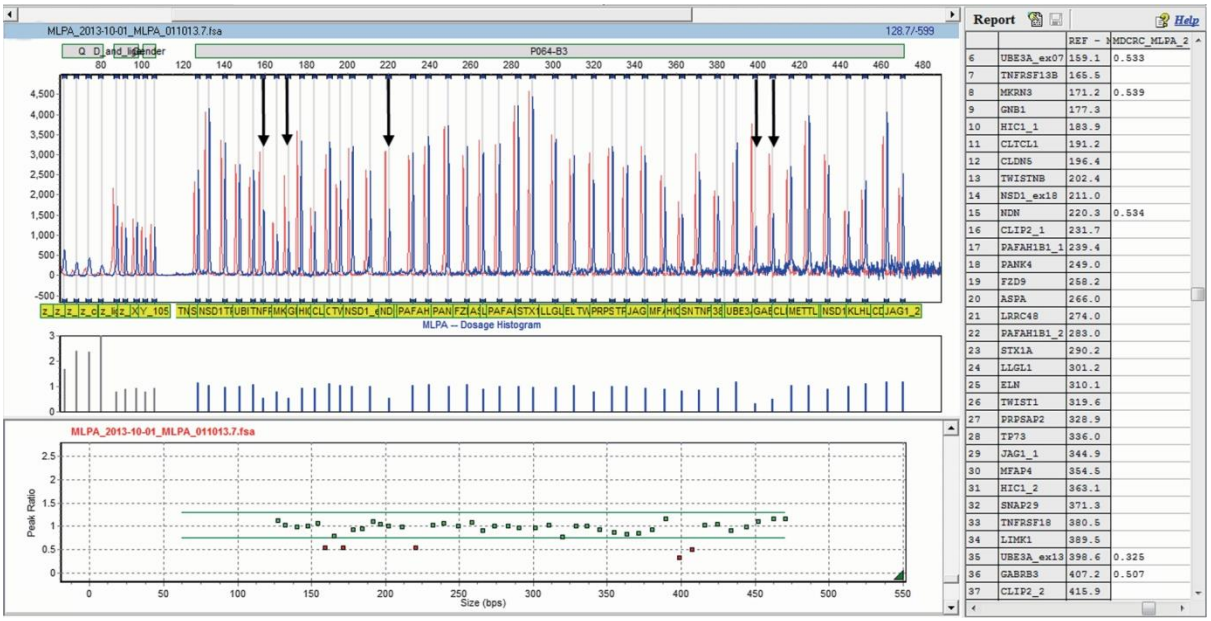

Fig 2: Subject M1. The ratios indicate a deletion for the five probes in the 15q11 region (deleted in Prader–Willi syndrome). MLPA peaks showing deletion/duplication of probes (arrows). Bottom panel and squares represent the ratio or gene dosage for each probe after normalization. Outliers (red squares) indicate a deletion or duplication. The ratios are tabulated on the right.

Table 2. Abnormal loci detected and ratios.

Individual	Type of abnormality	Region	Locus	Ratio
P064 kit				
M1	Deletion	15q11.2	*UBE3A*	0.533
	Deletion	15q11.2	*MKRN3*	0.539
	Deletion	15q11.2	*NDN*	0.534
	Deletion	15q11.2	*UBE3A*	0.325
	Deletion	15q11.2	*GABRB3*	0.507
M2	Duplication	7q11.23	*CLIP2*	1.349
	Duplication	7q11.23	*FZD9*	1.314
	Duplication	7q11.23	*STX1A*	1.316
	Duplication	7q11.23	*ELN*	1.428
	Duplication	7q11.23	*LIMK1*	1.464
	Duplication	7q11.23	*CLIP2*	1.482
P036 kit				
S1	Deletion	4pter	*PIGG*	0.588
S2	Deletion	17pter	*RPH3AL*	0.617
S3	Deletion	4pter	*PIGG*	0.543
	Duplication	8pter	*FBXO25*	1.486
S4	Deletion	6pter	*IRF4*	0.526
	Duplication	8pter	*FBXO25*	1.649
S5[a]	Duplication	3qter	*BDH1*	1.351
	Deletion	7qter	*VIPR2*	0.572
S6	Deletion	1qter	*SH3BP5L*	0.663
	Duplication (missed)	5pter	*PDCD6*	<1.3[b]
S7	Duplication (missed)	Ypter	*SHOX*	<1.3[b]
	Deletion	Yqter	*VAMP7*	0.591

Study included a male participant who was nine years old and exhibited developmental and speech delay. While speech delay is a common associated clinical finding in most patients with intellectual disability (ID), at least 25% of our study population showed delayed speech. These abnormalities were not apparent using conventional methods, highlighting the insufficiency of a diagnosis based solely on clinical examination or testing a few target loci. Our results emphasize that although common microdeletion syndromes have been described with characteristic features, the varying degrees of severity and complexity of ID necessitate a comprehensive genetic assessment of each individual.

CONCLUSION

Study highlights the importance of detecting genomic imbalances in children with intellectual disability (ID), especially those with no identifiable cause. We utilized the SALSA MLPA probe kit to detect interstitial and subtelomeric rearrangements in our study population and found a detection rate of over 98%. Our findings suggest that the SALSA MLPA probe kit is a highly effective method for detecting such imbalances. We also emphasize that a diagnosis based solely on clinical examination or testing a few target loci is insufficient, as our study participant with developmental and speech delay was not identified through conventional methods. Our results underscore the need for a comprehensive genetic assessment of each individual with ID, given the varying degrees of severity and complexity of the disorder. Overall, our study contributes to a better understanding of the genetic mechanisms underlying ID and could lead to improved diagnostic and therapeutic approaches, ultimately improving outcomes and quality of life for affected individuals and their families.

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