The use of Multiplex Ligation Dependent Probe Amplification (MLPA) in the detection of copy number variance of subtelomeric regions in idiopathic intellectual disability

Thesis

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List of Abbreviations

AAMR's	American Association on Mental Retardation's
ADHD	attention deficit disorder
ADID	Autosomal dominant intellectual disability
CNVs	copy number variants
CGH	comparative genomic hybridization
CMA	Chromosomal microarray
DNA	Deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition.
FISH	Fluorescence in situ hybridization
FMR1	fragile X mental retardation 1
FXS	Fragile X mental retardation syndrome
GDD	Global developmental delay
ID	Intellectual disability
IQ	intelligence quotient
LSI	Locus Specific probe
MAPH	multiplex amplifiable probe hybridisation
Mb	million base pairs

MCA	Multiple Congenital Anomalies
MLPA	multiplex ligation dependent probe amplification
MODY	Maturity-onset diabetes of the young.
NGS	Next generation sequencing
PCR	polymerase chain reaction
SNP	Single nucleotide polymorphism
Srpt	Subtelomeric repeat
STRP	Short tandem repeat polymorphisms
TORCH	Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections.
UCRs	Unbalanced the chromosomal rearrangements
XLID	X-linked intellectual disability
YAC	Yeast artificial clones

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Introduction

Intellectual disability, commonly referred to as mental retardation, is a frequent disorder associated with neurodevelopmental impairments in children ***(Shevell, 2008; García-Cazorla et al., 2009).*** The rate of intellectual disability prevalence is 1-3% depending on the population, criteria and sample methods applied ***(Moeschler and Shevell, 2006; Levy, 2011).*** In about 50% of the cases a specific causes can be identifed (***Shapiro and Batshaw, 2011)*** whereas in 30-50% the etiology is unknown this latter group is called idiopathic ***(Mandal et al., 2009)***.

Unbalanced chromosomal rearrangements (UCRs) are identified in a considerable proportion of patients as the cause of the observed intellectual disability with detection rates varying widely from 5 to 30% as reported in the literature ***(Roeleveld et al., 1997; Van Karnebeek et al., 2005).*** In particular, UCRs involving the distal ends or subtelomeric regions of chromosomes have been shown to be a significant cause of intellectual disability ***(Flint and Knight, 2003; Ravnan et al., 2006).*** Putatively based on the structural characteristics of chromosome ends and their role in the segregation process ***(Linardopoulou et al., 2005).*** Subtelomeric copy number variance could be the cause of 3-6% of idiopathic intellectual disability

(Ledbetter and Martin, 2007). The prevalence of subtelomeric copy number variance has been variable among reported different studies ranging from as low as zero in mildly affected cases to 30% depending on the inclusion criteria selected, which included dysmorphic features, congenital malformations and family history of abortions ***(Kriek et al., 2004; Medina et al., 2014).*** Subtelomeric regions are gene-rich. Many genes are likely involoved in small deletion in these regions, thus deleterious consequences result. Many of these subtelomeric deletions are now recognized as clinically recognizable phenotypes**.** These terminal regions stain lightly on karyotyping and deletion sizes are variable, which are often difficult to be detected by routine conventional examination ***(Battaglia, 2005)***.

Fluorescence in situ hybridization (FISH) was the first clinically used molecular cytogenetic technique ***(Pinkel et al., 1988)***. Subtelomeric FISH probe is the most commonly used technique in detecting subtelomeric copy number variance ***(Knight et al., 2000).*** They are commercially available and are fully tested by their manufacturer. Subtelomeric FISH probe is laborious, time-consuming and expensive procedures of using a complete set of subtelomeric FISH to analyze selected patients ***(Albert et al., 2006).*** Multiplex ligation-dependent

probe amplification (MLPA) technique have overcomed this limitation ***(Azofeifa et al., 2000; Boggula et al., 2014)***.

The Multiplex Ligation-Dependent Probe Amplification (MLPA) method is a molecular genetic technique that identifies subtelomeric copy number variance, such as deletions or duplications ***(Torrado et al., 2009; Medina et al., 2014).*** It is a new, sensitive, economical and simple method for relative quantification of multiple nucleic acid sequences in a single reaction. Introduced by MRC-Holland in January 2002, its principle relatively simple, in which denatured genomic DNA after standard extraction is hybridized with a mixture of standardized probes. Each MLPA probe consists of two oligonucleotides, the two parts of each probe hybridized to adjacent target sequences and are ligated by an enzyme (thermostable ligase). All probe ligation products are amplified by PCR using only one primer pair. Since the amplification product of each probe has a unique length, they can therefore be separated by electrophoresis. The relative amounts of probe amplification products reflect the relative quantity of target sequences ***(Lam et al., 2006) (refer to details in review of literature, chapter 3).***

Aim of the Work

The study aims to determine the prevalence, and characterization of copy number variance of subtelomeric regions through the multiplex ligation dependent probe amplification (MLPA) method in pediatric patients with idiopathic intellectual disability.