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Original Article

# Multiplex Ligation-Dependent Probe Amplification to Subtelomeric Rearrangements in Idiopathic Intellectual Disability in Colombia

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ABSTRACT

**BACKGROUND:** A cause cannot be determined in 30% to 50% of patients with intellectual disability. Determining the etiology of intellectual disability is important and useful for pediatric neurologists, geneticists, pediatricians, and patients' families because it allows assessment of recurrence risk, appropriate genetic counseling, and focus on treatment options and prognosis. This study aims to determine the prevalence, origin, and characterization of subtelomeric rearrangements through the Multiplex Ligation-Dependent Probe Amplification method in pediatric patients with idiopathic intellectual disability. **METHODS:** A cross-sectional descriptive study was undertaken with patients seen in consultation at the neuropediatrics or genetic service of the Central Military Hospital, the Mercy' Hospital, or the Genetics Institute National University of Colombia. Patients were diagnosed with idiopathic intellectual disability between December 2010 and September 2011 and underwent a complete medical history, physical examination, and assessment to rule out other etiologies of intellectual disability. Then we applied the genetic test of Multiplex Ligation-Dependent Probe Amplification to each patient's sample of peripheral blood to determine subtelomeric rearrangements. **RESULTS:** We studied a group of 119 patients with idiopathic intellectual disability; Multiplex Ligation-Dependent Probe Amplification showed subtelomeric rearrangements in five. In the group with subtelomeric rearrangements, the most frequent results were de novo rearrangements (80%), deletion type (60%), moderate and severe intellectual disability (80%), minor phenotypic abnormalities (80%), and family history of neurological disorders (80%). No dependence relationship was observed between subtelomeric rearrangements and family history of neurological disorders, family history of intellectual disability, severity of intellectual disability, phenotypic abnormalities, and consanguinity. **CONCLUSIONS:** This study determined a prevalence of subtelomeric rearrangements of 4.2% in a group of Colombian pediatric patients with idiopathic intellectual disability using the genetic test Multiplex Ligation-Dependent Probe Amplification.

***Keywords:*** intellectual disability, mental retardation, subtelomeric rearrangements, MLPA

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## Introduction

Intellectual disability, commonly referred to as mental retardation, is a frequent, typically chronic disorder associated with neurodevelopmental impairment in children.[1,2] The accepted rate of intellectual disability prevalence is 1% to 3% depending on the population, criteria, and sampling methods applied.[3-5] Specific causes are identifiable in about

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50% of the patients,[6] whereas in 30% to 50% the etiology is unknown.[7-9]

An important genetic cause of intellectual disability is subtelomeric rearrangements that have been studied since the 1990s.[10-13] Most subtelomeric regions are rich in genes and prone to recombinations,[14] causing increased gene diversity. The prevalence of subtelomeric aberrations has varied among different studies from as low as zero in mildly affected patients to 30% depending on the inclusion criteria selected, which in studies has included dysmorphic features, congenital malformations, and family history of abortions.[15-18] Subtelomeric arrangements could be the cause of 3% to 6% of idiopathic intellectual disability.[19] The Multiplex Ligation-Dependent Probe Amplification (MLPA) method is a molecular genetic technique that identifies subtelomeric rearrangements, such as deletions or duplications.[20-23] This study aims to determine the prevalence, origin, and characterization of subtelomeric rearrangements through the MLPA technique in a group of Colombian pediatric patients with idiopathic intellectual disability.

## Methodology

The three diagnostic criteria for intellectual disability established by the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, fourth edition, the American Association of Intellectual and Developmental Disabilities, and the Individuals with Disabilities Education Act are: an intelligence quotient of 70 or below, concurrent deficits in present adaptive functioning, and onset before 18 years of age. A cross-sectional descriptive study was done of patients with idiopathic intellectual disability seeking consultation at the neuropediatrics or genetic care service of the Central Military Hospital, the Mercy Hospital, or the Genetics Institute National University of Colombia, and diagnosed with idiopathic intellectual disability between December 2010 and September 2011. Prior authorization was given by the Ethics Committees of each institution. The inclusion criteria were age between 5 and 18 years and a confirmed diagnosis of idiopathic intellectual disability. Patients—or those responsible for patients—who did not agree to participate in the research were excluded, as were as those whose parents were not alive or not contacted. Children with intellectual disability underwent a complete medical history, including prenatal and postnatal history, physical examination, assessment of medical specialties, and such examinations as intellectual coefficient, conventional karyotyping, fragile X test, metabolic screening, neuroimaging, thyroid tests, and audiometry, depending on the characteristics of each patient and at the treating physician's discretion to rule out other etiologies of intellectual disability such as genetic, environmental, or acquired causes. Thus, we obtained a group of 119 children diagnosed with idiopathic intellectual disability. After signing the informed consent, patients provided a sample of 5 mL peripheral blood with the necessary aseptic precautions, which was later analyzed at the Genetics Institute National University of Colombia. This sample was used for DNA extraction with Ultra Clean DNA Blood Isolation Kit 100 purification from a sample of 300 μL blood, according to the manufacturer's instructions. The amplification and capillary electrophoresis was performed using MLPA MRC Holland for Telomeres kit (SALSA MLPA kit P070 Human Telomere-5), which contains probes for detecting deletions or duplications of each subtelomeric region. Analysis of the data obtained from capillary electrophoresis was done with the Coffalyser software recommended by the manufacturer of the MLPA kit (http://www.mrc-holland.com/WebForms/WebFormMain.aspx). Ratios of fluorescence with the control probes below 0.7 were considered as deletions and ratios above 1.3 as duplications. All patients who met this criterion were confirmed with the SALSA MLPA P036 Telomere-3 kit, which covers areas adjacent to the P070 kit used in this study. After the subtelomeric rearrangement was detected by the MLPA technique, blood samples from the patient's parents were also taken using two kits (P070 and P036 for result confirmation). The results were compared with information from free-access medical and genetic literature databases, thus determining the relation between the identified rearrangements and intellectual disability. The genetic test results were handed over to the parents or those responsible for the patients, who were advised to present them to the referring medical specialist. After the data were consolidated in an Excel worksheet, a descriptive ratio/percentage statistical analysis involving categorical or qualitative variables was performed. To identify possible relationships between the presence of subtelomeric rearrangements and perinatal or family factors, a chi-square test was done on 2 × 2 contingency tables.

**TABLE 1.**
Demographic characteristics

| Variable | Idiopathic Intellectual Disability | |
|---|---|---|
| | n | % |
| Age (yr) | | |
| 5-9 | 45 | 37.8 |
| 10-14 | 39 | 32.8 |
| 15-18 | 35 | 29.4 |
| Gender | | |
| Male | 69 | 58.0 |
| Female | 50 | 42.0 |
| Maternal age (yr) | | |
| 16-35 | 100 | 84.0 |
| 36-40 | 19 | 16.0 |
| Paternal age (yr) | | |
| 18-35 | 74 | 62.2 |
| 36-59 | 45 | 37.8 |
| Place of referral | | |
| Central Military Hospital | 52 | 43.7 |
| Mercy Hospital | 29 | 24.4 |
| Genetics Institute, National University of Colombia | 38 | 31.9 |
| Place of origin | | |
| Bogota | 83 | 69.8 |
| Cundinamarca | 9 | 7.6 |
| Tolima | 4 | 3.4 |
| Boyaca | 4 | 3.4 |
| Other matters | 19 | 16.0 |

## Results

The study group comprised 119 patients between the ages of 5 and 18 years diagnosed with idiopathic intellectual disability who referred from a neuropediatrics or genetics consultation at the Central Military Hospital, Mercy Hospital, and Genetics Institute National University of Colombia. One hundred percent of the patients were assessed by neuropediatrics specialists; according to their clinical features, several were referred to other specialists for a more specific evaluation: 74.0% to genetics, 67.2% to ophthalmology, and 36.1% to psychiatry, among others. An intellectual coefficient test was given to all patients, followed by other tests such as neuroimaging, audiometric, karyotype, and thyroid levels. Table 1 below shows the demographic characteristics of the study group.

All age groups were similar in number, and 57% were male. The place of origin with the highest rate was Bogota (69.7%). The MLPA results for the 119 patients studied showed that five individuals, unrelated to each other, with subtelomeric rearrangements—three males and two females—which represented a prevalence of 4.2% (Table 2). Table 3 compares patients with and without subtelomeric rearrangements.