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SCHOOL OF DOCTORAL STUDIES

Abstract Of PhD Thesis

Study of genetic mutations in patients with intellectual disability and multiple congenital anomalies

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Introduction

Intellectual disabilities are a heterogeneous group of neurodevelopmental disorders characterized by limitations in intellectual function and adaptive behaviour, with onset occurring during the developmental period. Since genetic factors are the main cause of neurodevelopmental disorders, efficient guidelines and screening strategies using molecular genetic techniques are necessary for Romanian patients with intellectual disability (ID) or global developmental delay (GDD) and multiple congenital anomalies (MCA). With the development of new molecular genetic techniques, such as Fluorescence In Situ Hybridization (FISH), Multiplex Ligation-dependent Probe Amplification (MLPA) and Comparative Genomic Hybridization (CGH), it became possible to identify genetic mutations such as copy number variations (CNVs) which have led to the understanding of the underlying mechanisms of neurodevelopmental disorders. Still, next-generation sequencing (NGS) analysis gives us more information on gene variants. Thus, CNVs are the primary cause of many recurrent microdeletion and microduplication syndromes. In addition, they represent the risk factor for other neurodevelopmental disorders such as autism spectrum disorders, schizophrenia, attention deficit and hyperactivity disorder, and others.

General objectives

The main objective of the thesis was to analyze and estimate the frequency of genetic mutations such as CNVs in pediatric patients with ID/GDD and MCA using the MLPA technique and CNVs' molecular characterization. Additionally, we aimed to confirm the positive cases using other MLPA follow-up kits, specific for the regions with detected CNVs, respectively, parental analysis. We also aimed to evaluate the clinical utility of the MLPA technique to identify the aetiology of ID in an average-equipped laboratory in Romania to optimise the molecular diagnosis and propose a testing algorithm for these patients. Another objective was to evaluate the cytogenetic, molecular and phenotypic characteristics in diagnosing ID/GDD and ACM through exceptional case studies and literature review.

Study 1. Study of CNVs by the MLPA technique in children with intellectual disability or global developmental delay and multiple congenital anomalies

The MLPA technique and data analysis of CNVs were performed in 142 patients included in the study with ID/GDD and MCA (patients with normal karyotype, with marker chromosomes and chromosomal heteromorphisms; patients with Prader-Willi, DiGeorge, and Williams syndromes were excluded). We used one of the SALSA® MLPA® kits, P064 and P245 (from MRC Holland), for specific microdeletion/microduplication syndromes. Depending on the detected mutation, positive cases were confirmed with MLPA follow-up kits. It was also possible to characterize chromosomal abnormalities (e.g., marker chromosomes). The initial detection rate of CNVs for "classical" microdeletion/microduplication syndromes in our study was 9.85% (14/142 patients), respectively 7.74% after the validation of 11 CNVs. Among these are Phelan-McDermid syndrome, Miller-Dieker syndrome, Koolen-de-Vries syndrome, microduplication syndromes of 15q11.2q13.1, 3q29, and 1p36.32 regions, and Emanuel syndrome. Statistical analysis revealed a significant correlation between CNVs and epilepsy, with no correlations between CNVs and the other assessed clinical characteristics. Parental analysis was possible in six cases with normal results, CNVs interpreted as *de novo*.

Study 2. Study of subtelomeric rearrangements by MLPA technique in patients with idiopathic ID or global developmental delay and literature review

In this study, we included 142 patients with idiopathic ID/GDD with/without MCA (patients with negative results in the first study, normal karyotype, with the presence of marker chromosomes and chromosomal heteromorphisms). We performed the MLPA technique to identify subtelomeric rearrangements using specific kits for subtelomeric regions. The validation of cases with identified CNVs was done with MLPA follow-up kits from MRC Holland. The detection rate of CNVs using the combination of MLPA P036 and P070 kits in our study was 13.38% (19 cases), of which 68.42% were microdeletions, respectively 9.85% confirmed cases (14 cases). Thus, the MLPA technique is a suitable approach for the first-line diagnosis of patients with idiopathic ID/GDD with/without MCA, with high efficiency and low cost. Statistical analysis revealed no significant correlation between subtelomeric rearrangements and demographic and clinical characteristics ($p < 0.05$). After performing the parental analysis, the maternal origin of the CNV was established in one case, and the rest of the CNVs were interpreted *de novo*.

Study 3. Cytogenetic, molecular and phenotypic characteristics of patients with ID and MCA: case studies

In this study, we evaluated the cytogenetic, molecular and phenotypic characteristics of five cases with ID/GDD with/without MCA and compared them with similar results in the literature using combinations of genetic tests. For case no. 1, we characterized the small supernumerary marker chromosome (sSMC) derived from chromosome 22, identified by karyotyping that characterizes Emanuel syndrome in a patient with ID and a complex congenital heart defect, using only combinations of MLPA kits. For case no. 2, by combining conventional analysis with aCGH analysis, we identify *de novo* 2q11.2q14.3 duplication in mosaicism in the heterozygous state derived from an sSMC. In the third case, a patient with craniosynostosis and ID, we identified the c.749C>G mutation of the *FGFR3* gene, a mutation specific to Muenke syndrome, by MLPA technique and Sanger sequencing analysis. Finally, for the last two cases, by using MLPA, aCGH and NGS analysis techniques, we identified and characterized the changes responsible for Pitt-Hopkins syndrome, respectively, OPHN1 syndrome in a family with ID and behaviour problems.

General conclusions

By applying stricter selection criteria of patients and a combination of MLPA kits (P245, P064, P036, P070), the cumulative diagnostic rate of the detected CNVs was 17.34% (30/173) cases with ID/GDD and MCA. Additionally, several MLPA follow-up kits allowed the identification and validation of rare chromosomal rearrangements. The most frequent CNVs detected involved the 22q13 chromosomal region, followed by the 17p13.3p13.3, 1p36.3, 15q11.2, 10q26.2q26.3 and 11q24.3q25 regions. There are no correlations between detected CNVs, demographic characteristics, and clinical features, except for epilepsy. We diagnosed the first case of microduplication 3q29 in a child from Romania. Also, for the first time in the literature, we described a patient with Phelan-McDermid syndrome and primary hyperparathyroidism, as well as a rare case of 18q21.2q22.1 microdeletion syndrome (Pitt-Hopkins syndrome), diagnosed by MLPA technique in combination with advanced molecular techniques (aCGH, NGS). Conventional cytogenetic analysis should not be neglected in the diagnosis of ID/GDD with/without associated congenital anomalies, which can detect structural chromosomal abnormalities. Moreover, advanced technologies based on microarrays (aCGH, SNP-array) or NGS cannot detect structural abnormalities but can characterize and determine the accurate size of the genetic mutations involved. Therefore, applying a combination of cytogenetic and molecular techniques contributes not only to the increase of the diagnosis rate among patients with ID and MCA but also to the appropriate clinical management and the prevention of new cases through accurate genetic counselling. In the absence of microarray-based technologies (aCGH, SNP-array), the MLPA technique is a “reliable” and inexpensive technique, easy to perform and interpret the results, which could be used as a first-line test for all patients with ID/GDD with/without MCA.

The thesis's originality

Considering the limited number of studies carried out in Romania regarding the analysis of genetic mutations such as CNVs in patients with ID and MCA by MLPA technique, our thesis has an increased note of originality by using several combinations of MLPA kits (P064, P245, P036, P070) and follow-up kits for a better molecular characterization of the detected CNVs, stricter selection criteria of patients in the studied groups and by conducting parental analysis. Another original aspect is represented by exceptional case studies with rare genetic mutations being the only cases published in Romania using the MLPA technique, such as the case of Emanuel syndrome. Applying complementary genetic investigations (NGS) allowed the identification of the variant with uncertain significance in the *NEDD4L* gene present in Pitt-Hopkins syndrome, possibly responsible for the complex phenotype in this patient. Furthermore, we described for the first time in the literature the case of a patient with Phelan-McDermid syndrome and primary hyperparathyroidism and 3q29 microduplication syndrome, among the few reported cases in the literature and the first described in Romania in a child. At the same time, the present work made it possible to establish an accurate genetic diagnosis for cases with identified genetic mutations and provide proper genetic counselling to their families. Therefore, in the absence of microarray-based techniques and financial limitations, we wanted to evaluate the usefulness of the MLPA technique and propose an algorithm for testing patients with neurodevelopmental disorders and associated congenital anomalies using combinations of MLPA kits, respectively the cytogenetic analysis to exclude structural abnormalities and mosaicisms which remains essential in the diagnosis of these patients.