

Copy Number Variations in Hereditary Spastic Paraplegia-Related Genes: Evaluation of an Iranian Hereditary Spastic Paraplegia Cohort and Literature Review

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Keywords

Copy number variation · GermlineCNVCaller · Hereditary spastic paraplegia · *SPAST* deletion · Whole-exome sequencing

Abstract

Introduction: In human genetic disorders, copy number variations (CNVs) are considered a considerable underlying cause. CNVs are generally detected by array-based methods but can also be discovered by read-depth analysis of whole-exome sequencing (WES) data. We performed WES-based CNV identification in a cohort of 35 Iranian families with hereditary spastic paraplegia (HSP) patients. **Methods:** Thirty-five patients whose routine single-nucleotide variants (SNVs) and insertion/deletion analyses from exome data were unrevealing underwent a pipeline of CNV analysis using the read-depth detection method. Subsequently, a comprehensive search about the existence of CNVs in all 84 known HSP-causing genes was carried out in all reported HSP cases, so far. **Results and Discussion:** CNV analysis of exome data indicated that 1 patient harbored a heterozygous deletion in exon 17 of the *SPAST* gene. Multiplex ligation-dependent probe amplification analysis confirmed

this deletion in the proband and his affected father. Literature review demonstrated that, to date, pathogenic CNVs have been identified in 30 out of 84 HSP-causing genes (~36%). However, CNVs in only 17 of these genes were specifically associated with the HSP phenotype. Among them, CNVs were more common in *L1CAM*, *PLP1*, *SPAST*, *SPG7*, *SPG11*, and *REEP1* genes. The identification of the CNV in 1 of our patients suggests that WES allows the detection of both SNVs and CNVs from a single method without additional costs and execution time. However, because of intrinsic issues of WES in the detection of large rearrangements, it may not yet be exploited to replace the CNV detection methods in standard clinical practice.

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Introduction

Copy number variations (CNVs) are deletions/duplications of DNA segments in the human genome which range from 1 kilobase to several megabases [Westland et al., 2015]. They play an essential role in evolution, diversity, and genetic traits [Hoyer et al., 2015; Günther

et al., 2016]. CNVs are formed by three primary mechanisms as follows: (i) nonallelic homologous recombination which can result in most of the recurrent CNVs, (ii) nonhomologous end-joining, and (iii) fork stalling and template switching models which lead to most of the nonrecurrent CNVs [Gu et al., 2008]. The occurrence of CNVs may be mediated by some repetitive elements of the human genome. In this regard, Alu elements as a family of short interspersed nuclear elements are frequently involved in the CNV formation due to their abundant distribution and sequence identity [Dridi, 2012; Kim et al., 2016]. These Alu-associated CNVs have been estimated to cause about 0.3% of human genetic diseases [Song et al., 2018]. Also, low copy repeats take an active part in the creation of CNVs by nonallelic homologous recombination [Liu et al., 2011].

Conventionally, the gold standard methods for the detection of CNVs have been multiplex ligation-dependent probe amplification (MLPA) and array-based comparative genomic hybridization; their main limitation is that they investigate the presence of CNVs in a low number of prespecified genes (in case of MLPA) or genome regions (in case of array-based comparative genomic hybridization) [Pös et al., 2021]. Massively, parallel next-generation sequencing (NGS) offers an appealing timely and economically alternative platform for the concurrent identification of single-nucleotide variants (SNVs) and CNVs [Viailly et al., 2021]. Although NGS-based methods including whole-exome sequencing (WES) are nowadays being widely used in the clinical setting for diagnosis purposes, the analysis process of exome data for CNV detection is yet challenging [Moreno-Cabrera et al., 2020; Zhao et al., 2020b]. In this context, an increasing number of tools have been developed to detect CNVs from exome data. However, the low concordance among the CNV variant callers indicates that these tools have not hitherto optimized for this goal [Zhao et al., 2020a].

Although CNVs are a large source of normal variations in the human genome, they may also be associated with several developmental and neurological disorders such as Alzheimer's disease (AD) [Cuccaro et al., 2017], autism spectrum disorders [Vicari et al., 2019], congenital heart defects [Xie et al., 2017], amyotrophic lateral sclerosis [Morello et al., 2018], and hereditary spastic paraplegia (HSP) [Bis-Brewer and Züchner, 2018].

HSP is a large group of inherited neurodegenerative disorders that are mainly characterized by progressive lower-limb spasticity and weakness and present a wide range of clinical and genetic heterogeneity [Bellofatto et al., 2019; Cui et al., 2020]. Clinically, there are pure and

complicated forms of HSP, depending on the absence or presence of additional symptoms such as cerebellar ataxia, peripheral neuropathy, retinal degeneration, mental impairment, epilepsy, dysarthria, dystonia, and Parkinsonism [Boutry et al., 2019; Mackay-Sim, 2021]. Thus far, 84 genes or loci have been associated with autosomal recessive (AR-HSP), autosomal dominant, X-linked, and mitochondrial inheritance patterns in HSP patients [Meyyazhagan and Orlicchio, 2022]. The exact incidence of CNVs in HSP is not yet apparent, but recent studies have revealed the critical role of CNVs in HSP; for instance, CNVs have been detected in ~8–41% of HSP type 4 (SPG4) patients, owing to the Alu genomic architecture of the *SPAST* gene [Boone et al., 2014]. Also, approximately 19% and 14% of, respectively, SPG11 and SPG7 patients have been reported to harbor CNVs in their associated genes due to the presence of several Alu-associated recombination hotspots [Sánchez-Ferrero et al., 2013; Günther et al., 2016]. Intriguingly, the complexity of the genomic architecture of the *PLP1* gene on chromosome X leads to the considerable occurrence of CNVs in patients with SPG2 and Pelizaeus-Merzbacher disease (PMD; ~80%) [Lee et al., 2006; Beck et al., 2015]. Given that no comprehensive study has been conducted on CNVs in Iranian HSP patients, we initially investigated the occurrence of CNVs in HSP-related genes in a cohort of Iranian HSP patients, and then we reviewed the pathogenic CNVs in all reported HSP cases.

Subjects and Methods

Editorial Policies and Ethical Consideration

This research was performed in the accordance with the Declaration of Helsinki and with the approval of the Ethics Board of the University of Social Welfare and Rehabilitation Sciences (USWR; IR.USWR.REC.1401.038) in Iran. Informed consent was signed by all the participants.

Patients

Seventy Iranian unrelated HSP families were referred to the genetic research center at USWR (2019–2021) for genetic analysis. WES was performed for each proband as a part of their diagnostic workup. Routine analysis of SNVs in WES data was done. In total, 35 patients, who did not reach a definite genetic diagnosis in their SNVs, were entered into a pipeline of CNV analysis.

CNV Detection Based on WES Data

WES was performed as previously described [Davarzani et al., 2022]. Genome Analysis Toolkit (GATK; v.4.0.10.1) was used to call CNVs. The DetermineGermlineContigPloidy module of GATK was used to determine autosomal and allosomal contig ploidy, and subsequently, the GermlineCNVCaller module was used to call CNVs. We explored the possible existence of CNVs in all the identified known HSP genes using the read-depth detection method [Benson et al., 2021].

CNV Confirmation by MLPA

To confirm the identified CNV in 1 of our patients, MLPA was carried out using the SALSA® MLPA® Probemix P165-C3 HSP mix-1 (MRC-Holland, Amsterdam, The Netherlands), according to the manufacturer's protocols. This Probemix contains 47 probes (16, 20, and 11 probes for the *ATL1*, *SPAST*, and reference genes, respectively), and it can detect large deletions or duplications in the *ATL1* and *SPAST* genes. Coffalyser.NET software was used for data analysis.

Search Method

Different subtypes of HSP and their related genes were searched in the OMIM database (<https://www.omim.org/>). Then, the existence of CNVs in all 84 known HSP-causing genes was interrogated. The literature review was done up to October 30, 2022, in PubMed, Scopus, ScienceDirect, and Google Scholar as well as in MalaCards (<https://www.malacards.org/>) and Human Gene Mutation Database (HGMD; <https://www.hgmd.cf.ac.uk/ac/index.php>) databases.

Results

Genetic Findings

Routine WES analysis identified the disease-causing SNVs in 35 probands [Khani et al., 2020; Hashemi et al., 2021; Pashaei et al., 2021; Rahimi Bidgoli et al., 2021; Davarzani et al., 2022; Hashemi et al., 2022; Ghasemi et al., 2023; Sadr et al., 2023a, b] (results of 10 families have not been published, yet). CNV analysis of HSP-causing genes for the remaining 35 probands identified the existence of CNV just in one proband (total 1/70; ~1.5% and 1/35; ~3% in this study). The proband CNV100-III5 carried a heterozygous deletion of exon 17, the final exon of the *SPAST* gene. MLPA analysis confirmed this heterozygous deletion in the proband and his affected father (Fig. 1a). Therefore, by CNV analysis, it was determined that SPG4 does run in this family (Fig. 1b).

Clinical Findings of the Proband CNV100-III5

The proband is a 37-year-old male (Fig. 1b) who was admitted to our center due to progressive difficulty of walking and stiffness of his lower limbs. The first symptoms began at the age of 22 years when he noticed walking more slowly. His symptoms worsened in cold weather. Some other individuals in his family also experienced gait problems (Fig. 1b). He denied sphincter dysfunction, and his cognition was normal. On neurological examination, he had a spastic gait, symmetrical proximal weakness, and mild spasticity of the lower limbs. Increased deep tendon reflexes and Babinski sign were present bilaterally. His spinal and brain magnetic resonance imaging, nerve conduction study, and visual evoked potential were normal.

Result of the Literature Review

A review of the literature revealed that pathogenic CNVs have been detected in 30 out of 84 HSP-causing genes (~36%), so far (online suppl. Table S1; for all online suppl. material, see <https://doi.org/10.1159/000531507>). Interestingly, CNVs in only 17 of these genes were specifically associated with the HSP phenotype (online suppl. Table S2). This literature review also unveiled that CNVs were more common in some of these genes including *L1CAM* (SPG1), *PLP1* (SPG2), *SPAST* (SPG4), *SPG7* (SPG7), *SPG11* (SPG11), and *REEP1* (SPG31). Below, we will briefly describe these genes with a focus on the importance of identified CNVs on HSP pathogenesis.

Autosomal Dominant HSPs

SPG4/SPAST (OMIM #182601)

SPG4 which is caused by variants in *SPAST* is the most frequent subtype of all HSP cases (~45%) and HSP patients with AD inheritance (~17–79%) [Boutry et al., 2019]. All types of variants including missense, nonsense, splice site alternations, small insertions/deletions (indels), gross deletions or duplication, and small complex rearrangements have been reported in HSP patients harboring *SPAST* variants [Boone et al., 2011; Lan et al., 2014]. Among them, CNVs significantly contribute to the HSP pathogenesis, accounting for ~8%–41% of SPG4 cases [Boone et al., 2014; Kadnikova et al., 2019]. Such CNVs primarily arise due to homologous recombination between nonallelic Alu elements that are highly concentrated in the introns and flanking sequence of *SPAST* (more three times than the genomic average). This preponderance of Alu-mediated CNVs suggests the Alu-rich genomic architecture of *SPAST* and the susceptibility of this gene to intragenic rearrangements. Therefore, these rearrangements can be a common cause of SPG4 [Boone et al., 2011, 2014].

Regarding the CNV found in 1 of the patients of our study, about 157 pathogenic CNVs (147 deletions and 10 duplications) have been reported, to date, in the *SPAST* gene (online suppl. Table S1). These CNVs present diversity in terms of size, genomic position, and rearranged exons emanating from different combinations of deleted exons [Depienne et al., 2007; Wang and Zhao, 2015]. Most of these combinations include the last exon of the *SPAST* gene, exon 17, which can lead to the fusion of *SPAST* and *SLC30A6*, its downstream gene. This fusion can alter the expression or transcript structure of the *SLC30A6* gene and consequently disrupts *SLC30A6* promoter elements or conveys a position effect. Considering the reported association between dysfunctional *SLC30A6* and the development of AD, the presence of dementia in some SPG4 cases with exon 17 deletion of *SPAST* could be

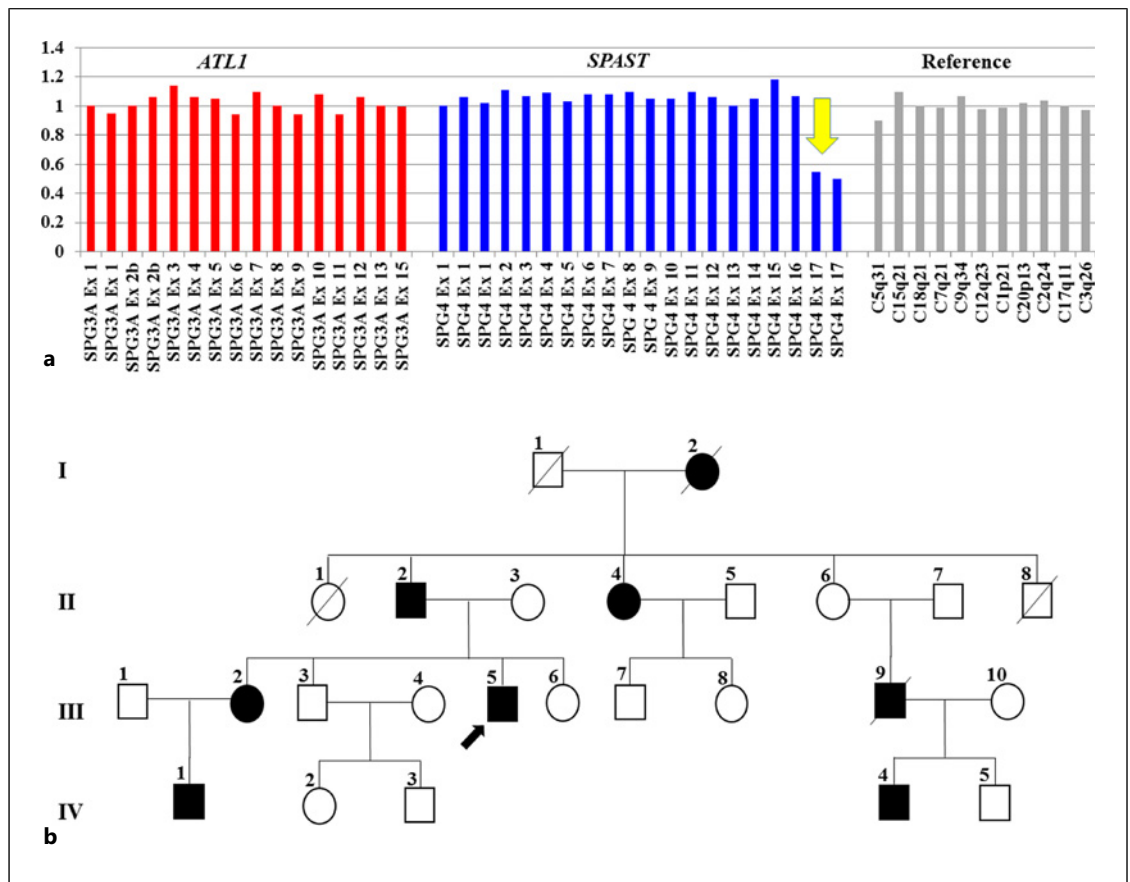


Fig. 1. a MLPA analysis of the *SPAST* and *ATLI* genes using the SALSA® MLPA® Probemix P165-C3 HSP mix-1 (MRC-Holland, Amsterdam, The Netherlands); this kit includes *ATLI* (red), *SPAST* (blue), and reference probes (gray). Heterozygous deletion of exon 17 is shown by the yellow arrow. **b** CNV100 pedigree with exon 17 deletion of the *SPAST* gene. The arrow denotes the proband. Blank circles and squares: healthy individuals; dark circles and squares: affected individuals.

explained [Boone et al., 2011, 2014]. Also, some deletions extend into an adjoining gene named *DPY30*, which is located upstream of *SPAST* in a head-to-head orientation. Haploinsufficiency of *SPAST* and *DPY30* can reduce the age at the onset in HSP patients [Newton et al., 2018]. The association between *SPAST* deletion and lower age of onset at the next generation was also marked in the study of Reddy et al. [2007]. Therefore, in our previous study, the observation of anticipation in an extended Iranian HSP family whose WES revealed no SNV prompted us to evaluate the existence of CNV in *SPAST* directly by MLPA, which resulted in the identification of a heterozygous large deletion of exon 17 in 8 HSP cases of the family [Hashemi et al., 2022]. Of interest, a report of multi-exonic SPG4 duplication in a large Brazilian pedigree demonstrated that some CNVs of *SPAST* may be related to sex-dependent penetrance of HSP [Mitne-Neto et al., 2007]. Like most previously reported HSP patients

with exon deletion in *SPAST*, our patients represented the pure HSP phenotype [Beetz et al., 2007; Depienne et al., 2007; Erichsen et al., 2007; Lu et al., 2014].

SPG31/*REEP1* (OMIM #610250)

REEP1 gene variants are the underlying cause of SPG31 [de Souza et al., 2017]. SPG31 is a relatively common subtype of HSP which has been identified in ~3% of all HSP cases [Salinas et al., 2008]. Different types of SNVs have been reported in *REEP1* [Lo Giudice et al., 2014]. However, compared to SNVs, the occurrence of CNVs seems to be rare in this gene [Beetz et al., 2008].

Autosomal Recessive HSPs

SPG11/*SPG11* (*KIAA1840*) (OMIM #604360)

SPG11, derived from variants of the *SPG11* (*KIAA1840*), is the most common subtype of AR-HSPs, accounting for

~20% of cases. Among several types of variants that lead to protein loss of function, pathogenic CNVs are responsible for almost 19% of all SPG11 cases [Günther et al., 2016]. The analysis of the *SPG11* gene indicates a high concentration of Alu elements that constitute a significant part of this gene (~101 kilobase). The presence of these elements results in intrinsic instability of the *SPG11*-containing locus and favors the occurrence of various gene rearrangements [Conceicao Pereira et al., 2012]. Of note, the reported CNVs of *SPG11* have been mostly related to the HSP phenotype (online suppl. Table S1).

SPG7/SPG7 (PGN) (OMIM #07259)

SPG7 stemming from *SPG7* (PGN) variants is the second most common form of AR-HSPs, being responsible for ~5% of AR-HSPs and ~12% of patients with sporadic spastic paraparesis negative for *SPAST* variants [Lopez et al., 2015; de Souza et al., 2017]. Pathogenic CNVs have been detected in 14% of SPG7 patients. These CNVs might be generated due to the induction of recombination by several Alu elements situated in this gene, especially at a typical 26-bp core sequence [Sánchez-Ferrero et al., 2013; Lopez et al., 2015]. By now, the reported CNVs in *SPG7* have been mostly associated with the HSP phenotype (online suppl. Table S1).

X-Linked HSPs

SPG2/*PLP1* (OMIM # 312920)

PLP1 variants can give rise to both SPG2 and PMD, two neurodegenerative disorders with different severity of the clinical course [Grossi et al., 2011]. Although CNVs in *PLP1* are a common cause of PMD, CNVs leading to deletions and duplications of *PLP1* are not frequently associated with HSP phenotypes (online suppl. Table S1).

SPG1/*LICAM* (OMIM # 303350)

In SPG1 patients, the two most prevalent types of reported *LICAM* variants have been missense and rearrangements [Kutsche et al., 2002; Xie et al., 2018]. The compact gene structure and the low density of repeats in the genomic *LICAM* DNA sequence predispose this gene to illegitimate recombination and consequent rearrangements including loss of the complete gene [Kutsche et al., 2002].

Discussion

In this study, by incorporating CNV detection into the routine workflow of exome data analysis, 1 of our HSP patients was found to be a heterozygote for the deletion of exon 17 in the *SPAST* gene. Implementing CNV detection

in the WES analysis pipeline increased the detection rate by ~1.5% (totally 1/70 and 1/35; ~3% in this study), which is in line with recent studies (up to 6%; an average of 2%) [Pfundt et al., 2017; Bergant et al., 2018; Dharmadhikari et al., 2019]. This indicates that for a more comprehensive molecular diagnostic assessment of clinically suspected HSP cases, integration of both CNVs and SNV/indel analyses would yield a relatively suited clinical utility. Concerning HSP, only two studies have adopted this strategy before in patients with HSP, one in a Kuwaiti-Jordanian family in whom WES failed to reveal any SNV or indel [Lee et al., 2017] and the other in 347 persons with a mostly pure HSP to find only KIF1A-associated SNVs or CNVs [Pennings et al., 2020]. In this respect, to the best of our knowledge, our study is the first work in which CNV analysis of a large cohort of HSP patients was performed by recruiting exome data. On the other hand, the results of our literature review demonstrated the relevance of CNVs in the etiopathogenesis of HSP. In this aspect, among HSP cases with AD inheritance, CNVs in the *SPAST* gene take a major part in SPG4 pathogenesis, which reflects that high enrichment Alu elements in this gene confer rearrangement susceptibility [Boone et al., 2014]. Of particular interest, our literature review indicated that among the known genes for AR-HSPs, the most reported CNVs in *SPG11* and *SPG7* have been mainly linked to the HSP phenotype. Also, CNVs in *PLP1* are not an important cause of X-linked SPG2, but CNVs in *LICAM* play a crucial role in the development of SPG1 [Kutsche et al., 2002; Xie et al., 2018]. Despite all these informative findings, the exact proportion of HSP cases caused by CNVs and the extent to which these CNVs implicate HSP pathophysiology are yet to be determined. This is because the pathogenic potential of CNVs can be contingent upon genetic ancestry and environmental factors [Nowakowska, 2017].

Currently, it is estimated that the diagnostic heritability gap in patients who are clinically suspected of HSP is 30–40%, even greater for sporadic cases [Bis-Brewer and Züchner, 2018]. Therefore, research into plausible genetic causes of HSP other than SNVs including CNVs could help to fill this diagnostic gap, at least in part. Also, the achievement of a precise genetic diagnosis would positively affect delivering genetic counseling to families with HSP as well as developing personalized medicine approaches for this disorder and other neurodegenerative diseases [Dilliot et al., 2022]. However, researchers are facing some challenges. For instance, CNVs emerged from Alu-mediated rearrangements could affect not only the involved HSP-related genes but also neighboring genes and extragenic areas, depending on the size,

genomic position, and rearranged exons [Boone et al., 2011]. This can lead to a wide range of phenotypic consequences, making an unclear genotype-phenotype correlation in HSP patients bearing CNVs [Wang and Zhao, 2015]. This phenomenon is exemplified very well in HSP patients with CNVs in *SPAST* [Boone et al., 2014].

Of importance, out of 35 SNV-negative HSP patients analyzed in this study, 34 remained genetically undiagnosed. We can theorize three scenarios for the underlying genetic causes of them. First, they may have SNVs in unknown genes for HSP. Second, SNVs may have occurred in intronic, regulatory, or untranslated regions which are not usually covered by WES. Third, they may harbor CNVs in yet unknown HSP genes. Hence, reanalysis of exome data for possible missed SNVs, performing WES for finding putative SNVs in uncovered regions by WES, and finally conducting high-resolution chromosomal microarray analysis for detecting CNVs in unknown HSP genes would probably lead to molecular diagnoses in our unsolved HSP patients.

Conclusion

In summary, our findings provide essential proof of the principle that exome-wide CNVs screening based on a read-depth detection method can slightly increase the diagnostic yield of routine WES analysis of HSP patients. This little improvement of diagnostic rate could be due to considerable current challenges in the identification of deletions or duplications from exome data. Albeit, with the improvement of software and algorithms in the future, CNV analysis from exome data can become a more suitable strategy because the combination of SNVs and CNV detection from a single source of data could allow a better cost-benefit and turnaround time. Finally, although the literature review informed us with some valuable information about the occurrence of CNVs in HSP-related genes, presently, we cannot reach a consensus about the molecular epidemiology of CNVs and CNVs-associated pathomechanism in HSP.

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Acknowledgments

We acknowledge the University of Social Welfare and Rehabilitation Sciences for funding the research (Grant No. 2592) and thank the patients and their family members for participating in the study.

Statement of Ethics

This research was performed in the accordance with the Declaration of Helsinki and with the approval of the Ethics Board of the University of Social Welfare and Rehabilitation Sciences (USWR; IR.USWR.REC.1401.038) in Iran. The participants were informed about the nature of the research, and written informed consent was obtained for participation in this study.

Conflicts of Interest Statement

All the authors claim the absence of financial interests and the absence of conflicts of interest.

Funding Sources

The University of Social Welfare and Rehabilitation Sciences (GRC/USWR; Grant No.-2592) provided funding for this study.

Author Contributions

Aida Ghasemi and Zahra Sadr: DNA extraction, whole-exome sequencing data analysis, MLPA analysis, and writing of manuscript; Mojgan Babanejad: CNV analysis and editing of manuscript; Mohammad Rohani: clinical evaluations and writing and editing of manuscript; Afagh Alavi: design and supervision of the research and writing and editing of the manuscript. All the authors read and approved the final version of manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Additional data are available on request from the corresponding author.

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