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Analysis of Genetic Testing Results in 93 Children with Unexplained Developmental Delay/Intellectual Disability (Post-print)

Authors: Wang Jing, Liu Yun, Huang Haoyu, Wu Jinting, Liu Chunming, Zhang Yangping, Wang Wenjuan

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Abstract

Background: Developmental delay (DD)/mental retardation (MR) has complex etiologies, diverse clinical manifestations, and strong heterogeneity, making early and accurate diagnosis of such children very difficult. Currently, there are few large-sample analyses of clinical data and genetic testing results for these children in China to provide counseling for definitive etiology and prognosis assessment. Objective: To analyze the genetic testing results of children with DD/MR to establish genetic diagnosis, formulate treatment plans, and evaluate prognosis. Methods: Ninety-three children with DD/MR of unknown etiology who visited the Rehabilitation Department of Kunming Children' s Hospital between September 2017 and September 2021 were selected for whole exome sequencing (WES) and copy number variation (CNV) detection to identify pathogenic gene mutation sites and CNV characteristics related to clinical manifestations, with Sanger sequencing used for proband verification to analyze the detection rate of gene mutations. Results: (1) All 93 children exhibited motor developmental delay, intellectual disability, or global developmental delay, with developmental levels behind normal developmental milestones; (2) Genetic variants were detected in 74 cases, with a detection rate of 79.5%, including 40 cases (43.1%) of pathogenic gene mutations, 13 cases (13.9%) of gene copy number variations, and 21 cases with variants of uncertain significance; (3) Over 50 genes were involved, with spinal muscular atrophy caused by SMN1 gene mutations accounting for the highest proportion (4/40) among the resulting diseases, followed by Bethlem myopathy type 1 caused by COL6A2 gene mutations (3/40) and Joubert syndrome type 21 caused by CSPP1 gene mutations (2/40). Conclusion: Pathogenic gene mutations and copy number variations may be the main causes of DD/MR. The use of WES combined with CNV provides a basis for clarifying the etiology of DD/MR, which is particularly important for children with atypical diagnostic phenotypes and clinical manifestations.

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Full Text

Analysis of Genetic Test Results in 93 Children with Unexplained Developmental Delay/Mental Retardation

WANG Jing, LIU Yun*, HUANG Haoyu, WU Jinting, LIU Chunming, ZHANG Yangping, WANG Wenjuan

Department of Rehabilitation, Kunming Children' s Hospital, Yunnan 650034, China

Corresponding author: LIU Yun, Chief Physician, E-mail: liuyun@etyy.cn

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Abstract

Background: Developmental delay (DD)/mental retardation (MR) has a complex etiology with diverse and heterogeneous clinical presentations, making early and accurate diagnosis extremely challenging. Currently, few large-scale studies in China have analyzed the clinical data and genetic test results of this patient population to provide counseling on definitive etiology and prognosis assessment.

Objective: To analyze genetic testing results in children with DD/MR of unknown origin to establish genetic diagnoses, guide treatment planning, and predict prognosis.

Methods: We enrolled 93 children with unexplained DD/MR who attended the Rehabilitation Department of Kunming Children's Hospital between September 2017 and September 2021. Whole-exome sequencing (WES) and copy number variation (CNV) testing were performed to identify pathogenic mutation loci and copy number variants associated with clinical manifestations. Sanger sequencing was used for proband validation, and mutation detection rates were analyzed.

Results: (1) All 93 children exhibited motor developmental delay, intellectual disability, or global developmental delay, with developmental levels behind normal milestones. (2) Genetic variants were detected in 74 cases, yielding a detection rate of 79.5%. Pathogenic gene mutations were identified in 40 cases (43.1%), gene copy number variants in 13 cases (13.9%), and variants of uncertain significance in 21 cases. (3) Over 50 genes were implicated. The most

common disease was spinal muscular atrophy caused by SMN1 gene mutation (4/40), followed by Bethlem myopathy type 1 caused by COL6A2 gene mutation (3/40) and Joubert syndrome type 21 caused by CSPP1 gene mutation (2/40).

Conclusion: Pathogenic gene mutations and copy number variants may be the main causes of DD/MR. The combined WES and CNV approach provides a basis for clarifying the etiology of DD/MR, particularly for children with atypical phenotypes and clinical presentations.

Keywords: developmental delay; mental retardation; whole exome; copy number variation; genetic testing

Introduction

Developmental delay (DD)/mental retardation (MR) is a common condition in pediatric rehabilitation. DD refers to significant deficits in adaptive functioning across social interaction, motor skills, cognition, and language, with delays in motor, language, or cognitive development compared to normal developmental milestones. It may involve a single domain, such as motor, cognitive, or language delay, or multiple domains causing global developmental delay (GDD) [1]. MR occurs in children under 18 years of age and is characterized primarily by intellectual disability and inadequate social adaptation [2]. MR is typically used to diagnose children aged $ $5 years with intellectual disability, while DD/GDD is used for children under 5 who show developmental delays in single or multiple domains of motor, language, and cognition that cannot yet be assessed through standardized intelligence testing. This includes children too young for standardized testing who require dynamic and comprehensive evaluation; some may not meet MR diagnostic criteria as they grow older [3]. The WHO reports the incidence of DD/MR as 1–3% [4]. The etiology is complex, involving not only genetic and environmental factors but also endocrine abnormalities and perinatal factors, with diverse and heterogeneous clinical manifestations that pose challenges for early and accurate diagnosis [5]. Whole-exome sequencing (WES) and copy number variation (CNV) testing are increasingly used for genetic diagnosis in these children. This study analyzed the clinical symptoms and genetic mutations in 93 children with unexplained DD/MR who attended the Rehabilitation Department of Kunming Children' s Hospital between September 2017 and September 2021, aiming to provide a theoretical basis for clinical diagnosis of DD/MR of unknown origin.

Methods

Study Subjects

We selected 93 children diagnosed with unexplained DD/MR who attended the Rehabilitation Department of Kunming Children's Hospital between September 2017 and September 2021. Developmental level was assessed using the following

methods [6]: children under 5 years were evaluated using the Child Neuropsychological and Behavioral Assessment Scale, while those aged 5 years and older were assessed using the Chinese Wechsler Intelligence Scale to determine developmental quotient (DQ) or intelligence quotient (IQ). Social adaptive functioning was evaluated using the Infant-Junior High School Student Social Adaptive Ability Scale. Diagnostic criteria: DD/MR was diagnosed when IQ/DQ scores were <70 and accompanied by low social adaptive ability.

Inclusion criteria: Clinical manifestations met at least one of the following: (1) developmental delay in motor, language, or cognitive skills, or global developmental delay; (2) dysmorphic facial features.

Exclusion criteria: Cases with perinatal infection, postnatal central nervous system infection, hypoxia, birth trauma, prematurity, known genetic syndromes, common genetic metabolic diseases, or those who refused genetic testing or were lost to follow-up were excluded.

A total of 93 children were enrolled, including 54 males and 39 females, with ages ranging from 8 months to 11 years and 2 months (mean age: 5.06 ± 2.34 years). This study was approved by the Medical Ethics Committee of Kunming Children' s Hospital (Approval No.: 2017-03-269-K01), and informed consent was obtained from all guardians.

Data Collection

We collected clinical data including medical history, developmental assessment scale scores, imaging studies, electroencephalogram (EEG) results, and other auxiliary examinations.

Genetic Testing Methods

Peripheral venous blood (2–4 mL) was collected in anticoagulant tubes. DNA was extracted using a blood genome extraction kit (Cowin Biotech), and sample quality was monitored using a fluorometer and agarose gel electrophoresis. The IDT xGen® Exome Research Panel v1.0 capture probe was used to construct whole-exome and genome databases, with sequencing depth >120× and coverage of 1×. Second-generation high-throughput sequencing was performed on the Illumina platform at the Zhiyin Oriental Translational Medicine Research Center. Detected CNV variants were classified according to the 2020 guidelines issued by the American College of Medical Genetics and Genomics (ACMG) [7].

Neurodevelopmental Assessment Methods

All enrolled children underwent neurodevelopmental assessment conducted uniformly by professional developmental assessment physicians in our department. Children under 5 years were assessed using the Child Neuropsychological and Behavioral Assessment Scale (abbreviated as "Er Xin Scale"), while those aged 5 years and older were evaluated using the Chinese Wechsler Intelligence Scale

to determine developmental quotient (DQ) or intelligence quotient (IQ). Social adaptive ability was determined using the Infant-Junior High School Student Social Adaptive Ability Scale. DQ/IQ scores of 50–70 indicated mild developmental delay, 35–49 indicated moderate delay, and <34 indicated severe delay [6].

Results

General Data

Among the 93 children, 76 (81.7%) were diagnosed with DD and 17 (18.2%) with MR. There were 54 males (58%) and 39 females (42.2%). Age at onset was 6 months to 1 year in 29 cases (31.2%), 1–3 years in 47 cases (50.5%), and $ $3 years in 17 cases (18.2%). The mean age was 35.61 ± 14.53 months. All children exhibited motor developmental delay, intellectual disability, or global developmental delay.

Neurodevelopmental Assessment Results

Neurodevelopmental assessment revealed mild to moderate developmental delay in 43 cases (46.2%), including 25 males (26.8%) and 18 females (19.3%). Severe delay was observed in 38 cases (40.8%), including 29 males (31.1%) and 9 females (9.7%).

Genetic Analysis

Genetic variants were detected in 74 of the 93 children, yielding a detection rate of 79.5%. Pathogenic gene mutations were identified in 40 cases (43.01%), gene copy number variants in 13 cases (13.9%), and variants of uncertain significance in 21 cases. Among the 40 children with pathogenic gene mutations, 27 were male (67.5%) and 13 were female (32.5%). Age distribution was 6 months to 1 year in 10 cases (25%), 1–3 years in 20 cases (50%), and $ $3 years in 10 cases (25%).

Clinical Phenotypes of Pathogenic Variants

Among the 40 children with pathogenic variants, 25 exhibited congenital anomalies such as dysmorphic facial features and transverse palmar creases, 9 had recurrent seizures, and 17 showed abnormal EEG results. All 40 children demonstrated varying degrees of developmental delay: mild in 6 cases, moderate in 23 cases, and severe in 11 cases (Table 1).

Genetic Findings

Genetic analysis of the 93 children identified variants in the following genes: SMN1 (n=4), COL6A2 (n=3), CSPP1 (n=2), MECP2, FOXG1, G6PD, LAMA2, GNB1, SHANK3, CHKB, KIAA0195, SPEG, RPGRIP1, CABP4,

MCM3AP, SPR, GNAO1, SMARCA4, SYT1, CTCF, PAX3, CSNK2A1, TRIP12, PUF60, EP300, CNOT3, TCF4, NR2F1, NFIX, COL1A1, COL12A1, SPTAN1, FLNB, CHD8, AIFM1, SOS1, DVL1, NLGN3, RYR1, chrX, ALG8, AFF2, NLGN4X, QARS1, UBE3A, AGRN, CACNA1H, CABP4, OCA2, and CACNA1C.

The most common condition was spinal muscular atrophy caused by SMN1 gene mutation (4/40), followed by Bethlem syndrome type 1 caused by COL6A2 gene mutation (3/40) and Joubert syndrome type 21 caused by CSPP1 gene mutation (2/40) (Table 2).

The genomic loci of the 13 cases (15.5%) with gene copy number variants are shown in Table 3 .

Discussion

DD/MR is a common developmental disorder in children, with cognitive function and social adaptive abilities significantly 落后于同龄正常儿童 lagging behind those of typically developing peers [8]. Literature reports indicate that genetic factors account for 17.4–47.1% of DD/MR etiologies [9], consistent with our diagnostic yield of 43.1%. Diagnosing children with unexplained DD/MR is particularly difficult, especially in those without distinctive clinical phenotypes, dysmorphic features, or congenital disabilities, often leading to missed or misdiagnosis. Early intervention is crucial for children with DD/MR, as it can minimize functional impairments and negative impacts on growth and development, and help children reintegrate into family and society [10]. However, only a minority of children receive early detection, identification, and intervention. Most children with developmental delay appear normal at birth, with delays becoming apparent during development, causing them to miss the optimal intervention window. Therefore, early detection and diagnosis are essential for children with DD/MR.

The symptoms of DD/MR are complex with substantial phenotypic heterogeneity, making it difficult to determine associations with gene mutations or chromosomal abnormalities based on a single characteristic clinical manifestation. Multiple factors influence clinical presentation, including mutation site function, deletion fragment size, and genes contained within deleted fragments. Numerous gene mutations and copy number variants associated with DD/MR remain undiscovered, and identified variants require further study [11]. Therefore, large-scale genetic studies are needed to identify genetic markers that may cause DD/MR. Genetic testing has been applied to diagnose the genetic etiology of DD/MR without clear causes, with U.S. guidelines identifying it as part of the standardized diagnostic workflow. WES can detect single gene site changes, providing evidence for diagnosing monogenic DD/MR, while CNV can detect copy number gains, losses, and inversions to clarify relationships with clinical phenotypes.

Over the past three years, 296 children with DD/MR have attended our hos-

pital' s rehabilitation department. Analysis of birth history, growth and development history, and auxiliary examinations identified etiology-related causes in 128 cases, primarily including premature low birth weight, neonatal hypoxic asphyxia, hypoxic-ischemic encephalopathy (HIE), hyperbilirubinemia, intracranial infection or hemorrhage, cortical malformation, autism spectrum disorder, and hypothyroidism. This study performed genetic testing on 93 children with unexplained DD/MR who consented to testing, revealing higher detection rates for spinal muscular atrophy, Bethlem syndrome type 1, and Joubert syndrome type 21.

Spinal muscular atrophy is an autosomal recessive neurodegenerative disease caused by survival motor neuron gene defects leading to dysfunction of anterior horn cells in the spinal cord, resulting in progressive muscle atrophy and weakness [12]. Bethlem syndrome is an autosomal dominant myopathy caused by COL6A1 gene variants, characterized primarily by proximal muscle weakness, long finger flexor contractures, and joint contractures at the elbows and ankles [13]. Joubert syndrome is a rare neurological disorder commonly presenting with neonatal episodic apnea, cerebellar vermis hypoplasia, muscle weakness, and developmental delay. Literature has reported 34 associated pathogenic genes, with CSPP1 being the 21st identified [14].

Among the 40 children with pathogenic variants, 25 had congenital anomalies such as dysmorphic facial features, including bilateral transverse palmar creases, cryptorchidism, low-set ears, small teeth, flat facial profile, depressed nasal bridge, strabismus, cardiac disease, overlapping toes/fingers, genu valgum, auricular deformity, sparse hair, concomitant exotropia, and nystagmus. Nine children had recurrent seizures, and 17 showed abnormal EEG results. All children demonstrated varying degrees of developmental delay. We also observed that different children may have deletions or duplications of the same gene segment or identical gene mutations, yet show phenotypic differences due to clinical heterogeneity. Diseases associated with copy number variant loci include cri-du-chat syndrome, Angelman syndrome, Williams-Beuren syndrome, Prader-Willi syndrome, Phelan-McDermid syndrome, and Potocki-Lupski syndrome.

In conclusion, accurate etiological diagnosis of DD/MR is the prerequisite for effective clinical rehabilitation, maximizing reduction of functional impairment and improving prognosis while preventing birth of children with DD/MR. Gene mutations and copy number variants are the main genetic causes of DD/MR. The combined WES and CNV approach provides a basis for clarifying the etiology of DD/MR and enables targeted treatment and genetic counseling based on test results, improving therapeutic efficacy and prognosis. Future studies should further employ WES combined with CNV to identify novel candidate genes for DD/MR, establish genetic diagnoses, develop treatment plans, and predict prognosis.

Author Contributions: WANG Jing and LIU Yun conceived the study, de-

signed the research protocol, and took overall responsibility for the article; HUANG Haoyu and WU Jinting conducted experiments and established subject selection and inclusion/exclusion criteria; LIU Chunming and WANG Wenjuan collected data, performed statistical analysis, retrieved literature, and prepared figures and tables; WANG Jing and ZHANG Yangping drafted the manuscript and analyzed and interpreted the main findings; LIU Yun revised the final version.

Conflict of Interest: The authors declare no conflict of interest.

References

[1] LIU Y, MA J, HE X M, et al. Current status and recommendations on the naming of developmental disorders associated with rehabilitation in children[J]. Chinese General Medicine, 2022, 25(08): 899-904. DOI: 10.12114/j.issn.1007-9572.2022.01.301.

[2] HUANG H, XU S S. Diagnosis and early intervention of mental retardation in infants and young children[J]. Chinese Journal of Practical Pediatric Clinics, 2013, 6(11): 879-880. DOI: 10.3760/cma.j.issn.2095-428X.2013.11.025.

[3] Topcu M, Yalnizoğlu D. Developmental abnormalities and mental retardation: diagnostic strategy[J]. Handb Clin Neurol. 2013;111:211-7. DOI: 10.1016/B978-0-444-52891-9.00021-X.

[4] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: DSM-5 [EB/OL] . (2013-05-22) [2019-08-08] . http://www.psych.org.2013/05/22.

[5] Bowling KM, Thompson ML, Amaral MD, et al. Genomic diagnosis for children with intellectual disability and/or developmental delay[J]. Genome Med. 2017, 9(1): 43. DOI: 10.1186/s13073-017-0433-1.

[6] The Neurology Group of the Chinese Pediatric Society of the Chinese Medical Association, the Child Neurology Professional Committee of the Chinese Medical Doctor Association Neurology Branch. Expert consensus on etiological diagnosis strategies for children with intellectual disability or global developmental delay[J]. Chinese Journal of Pediatrics, 2018, 56(11): 806-810. DOI: 10.3760/cma.j.issn.0578-1310.2018.11.003.

[7] Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen)[J]. Genet Med, 2020, 22(2): 245-257. DOI: 10.1038/s41436-019-0686-8.

[8] Vasudevan P, Suri M. A clinical approach to developmental delay and intellectual disability[J]. Clin Med (Lond). 2017, 17(6): 558-561. DOI: 10.7861/clinmedicine.17-6-558.

[9] Battaglia A, Doccini V, Bernardini L, et al. Confirmation of chromosomal microarray as a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, autism spectrum disorders and dysmorphic features[J]. Eur J Paediatr Neurol. 2013, 17(6): 589-99. DOI: 10.1016/j.ejpn.2013.04.010.

[10] Monica Juneeja, Rahul Jain, Biswaroop Chakrabarty, et al. Indian Children with Developmental Disability: Early versus Late Referral for Intervention[J]. Indian J Pediatr, 2014, 81(11): 1171-1178. DOI: 10.1007/s12098-014-1344-4.

[11] TENG ZT, WANG H. Advances in the molecular genetics of mental retardation or developmental delay[J]. International Journal of Pediatrics, 2018, 45(10): 768-771. DOI: 10.3760/cma.j.issn.1673-4408.2018.10.007.

[12] WU X, LI M. Progress in the study of spinal muscular atrophy phenotype and related genes[J]. New Clinical Medicine in China, 2021, 14(07): 725-729. DOI: 10.3969/j.issn.1674-3806.2021.07.21.

[13] WU RH, QIU KY, TANG WT, et al. A case of Bethlem myopathy with chimeric mutations in the COL6A1 gene and review of the literature[J]. New Medicine, 2021, 52(12): 941-946. DOI: 10.3969/j.issn.0253-9802.2021.12.010.

[14] Radha Rama Devi A, Naushad SM, Lingappa L. Clinical and Molecular Diagnosis of Joubert Syndrome Related Disorders[J]. Pediatr Neurol, 2020, 106(05): 43-49. DOI: 32139166.10.1016/j.pediatrneurol.2020.01.012.

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