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ORIGINAL RESEARCH

Whole-Exome Sequencing for Identifying Genetic Causes of Intellectual Developmental Disorders

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Background: Intellectual developmental disorders (IDD) generally refers to the persistent impairment of cognitive activities and mental retardation caused by physical damage to the brain or incomplete brain development. We aimed to explore its genetic causes.

Methods: In this study, 21 IDD patients were recruited. The Gesell developmental scales (GDS) and Wechsler intelligence scale for children (WISC) were used to assess the impaired level of intellectual development for all probands. A superconducting MRI scanner (Philips AcsNT 3.0 T Philips, Best, The Netherlands) was used to perform a plain MRI scan of the skull on the probands. The whole-exome sequencing was carried out using next-generation sequencing in all probands and their families.

Results: Eight had seizures and four had typical characteristics of autism. Pregnancy and delivery were uneventful except for three patients. Moderate IDD (52.4%) accounted for the majority. The abnormal MRI results included ventriculomegaly, pachygyria, broadening external cerebral space, abnormal signal change and agenesis of corpus callosum. Eleven variants were identified, including the variant in *CREBBP, MECP2, HCFC1, ATRX, RAB39B, CLCN4, DYRK1A* and *CASK*genes. The function areas result of gene-positive group were compared to that of gene-negative group. Not significant ($p>0.05$) items were revealed after this analysis.

Conclusion: Eleven variants were identified, including the variant in *CREBBP, MECP2, HCFC1, ATRX, RAB39B, CLCN4, DYRK1A* and *CASK* genes. The function areas result of gene-positive group were not significantly different from the gene-negative group.

Keywords: intellectual developmental disorders, gene variant, whole-exome sequencing

Introduction

Intellectual developmental disorders (IDD) generally refers to the persistent impairment of cognitive activities and mental retardation caused by physical damage to the brain or incomplete brain development.[1] As a result of genetic variation, infection, poisoning, head injury, craniocerebral malformation or endocrine abnormalities and other harmful factors, the brain of the fetus or infant cannot develop normally or completely, so that the development of intellectual activities stays at a relatively low stage.[2]

The intelligence of children with IDD is significantly lower than the average intelligence level of normal people.[3] The average intelligence quotient (IQ) of a normal person is 100. A child with an IQ of 100 is considered normal, and a child with an IQ below 70 is said to be "significantly below" the average (simplified to "below 70").[4] Only two out of 100 children of the same age had IQ below 70.[5] The onset of mental retardation is usually in the stage of development, specifically before

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the age of 18.[6] This distinguishes mental retardation that occurs during development from mental retardation that occurs later in life for a variety of reasons.[7] The incidence of mental retardation generally does not exceed 2%. Some children with mental retardation are accompanied by abnormal behaviors and mental diseases to a certain extent, which will also affect their daily social life.[8] According to the new development trend, people pay more and more attention to the social adjustment disorder of children with intellectual disabilities, because the social adjustment disorder has a direct impact on their personal function and how to participate in social life.[9] Children with IDD have obvious obstacles in adapting to daily social life. Young children with mental retardation in daily life for action, language development, not interpersonal communication, kindergarten or primary school is more difficult. In this study, 21 IDD patients were recruited to explore its genetic causes.

Materials and Methods

Ethical Compliance

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Guangdong Provincial People's Hospital. The parents of the patient in Figure 1 provided informed consent for the images to be published.

Subjects

In this study, 21 patients with intellectual developmental disorders (IDD) from the Department of Pediatrics, Guangdong Provincial People's Hospital were recruited. The inclusion criteria were: 1) age at first exam was from 3months to 18years; 2) IDD:ID: IQ<70, assessed by WISC; DD: DQ<76 in two or more developmental domains assessed by Gesell Developmental Scale. And patients with the history of intoxication, cranial trauma or central nervous system infection were excluded. Intellectual developmental disorder was defined as impaired intellectual functioning and adaptive behaviour with an onset before 18 years old. The study was approved by the Ethics Committee of Guangdong Provincial People's Hospital. Informed consent was obtained from the parents. The Gesell developmental scales (GDS) and Wechsler intelligence scale for children (WISC) were used to assess the impaired level of intellectual development for all probands. A superconducting MRI scanner (Philips AcsNT 3.0 T Philips, Best, The Netherlands) was used to perform a plain MRI scan of the skull on the probands.

Molecular Analysis

The whole-exome sequencing was carried out using next-generation sequencing (NGS) in all probands and their families. Genomic DNA was obtained from peripheral blood cells by standard procedures. DNA samples were extracted from blood samples using the Genomic DNA Extraction Kit (TIANGEN, Beijing, China), according to the manufacturer's protocol. The NGS procedure included ultrasonic fragmentation of DNA fragments, library construction, hybridisation capture, capture library amplification and purification and other standard procedures. Exomes were enriched with a use of the SureSelect Target Enrichment System Kit (Agilent, USA). The hybridisation capture for genomic DNA library was prepared using the Sureselect XT Reagent Kit. Targeted NGS including exon capture and sequencing on Genome Analyzer II platform (Illumina, Inc., San Diego, CA, USA) was performed for each index patient. Subsequently, the HGMD database (http://www.hgmd.cf.ac.uk/ac/index.php), dbSNP database (https://www.ncbi.nlm.nih.gov/snp/), gnomAD database (https://gnomad.broadinstitute.org/), and 1000 Genome database (https://www.internationalgenome.org/) were used for annotating the detected variations. Variant pathogenicity was

Figure 1 Facial and limb features of the patient.

analyzed in silico tool Mutation taster, Sorts Intolerant From Tolerant (SIFT), Polyphen2, Provean, M-CAP, REVEL. The pathogenicity of gene variation was analyzed according to American College of Medical Genetics and Genomics (ACMG) guidelines.

Statistical Methods

The descriptive statistics were used to present the clinical characteristics of the patients. The GDS assessment results were presented as developmental quotient (DQ). The intelligence quotient (IQ) of 4 children with IDD was assessed with Wechsler scale. In the intelligence development assessment (Gesell results), the results of gene-positive group were compared to that of gene-negative group. Using a two-tailed paired *T*-test. An alpha level of 0.05 (two-tailed) was our threshold of statistical significance.

Results

The Clinical Features of 21 Patients with Intellectual Developmental Disorders

As shown in Table 1, the male patients (85.7%) accounted for a large proportion of the probands. Eight of them with seizures were identified and four of them had typical characteristics of autism, including the three female patients. Table 1 shows the clinical features of each patient. Pregnancy and delivery were uneventful except for three patients (proband #2,11,12). The degree of IDD ranges from mild to profound. And moderate IDD (52.4%) accounted for the majority. Brain MRI showed normal except for eight patients. The abnormal MRI results included ventriculomegaly, pachygyria, broadening external cerebral space, abnormal signal change and agenesis of corpus callosum.

The Gene Variants in 11 Children with Intellectual Developmental Disorders

Among the 21 patients, 11 variants were identified, including the variant in *CREBBP* (Figures 1 and 2), *MECP2, HCFC1, ATRX, RAB39B, CLCN4, DYRK1A* and *CASK* genes (Table 2). The *MECP2* gene variants and *HCFC1* variants were found two or more times. Six de novo variants (28.6%) and 5 hemizygous variants (23.8%) were identified. Most variants were missense variants.

Comparison of Gesell Results Between Gene Positive and Negative Group

As shown in Tables 3 and 4, of the 21 patients, the intelligence quotient of 4 patients with IDD was assessed with Wechsler scale and 17 patients was assessed with Gesell scale. A preliminary global analysis of the Gesell results was performed. Analysis of Gesell developmental scales showed that function areas, including adaptation, gross motor, fine motor, individual communication, and language development. The function areas results of gene-positive group were compared to that of gene-negative group. Not significant ($p>0.05$) items were revealed after this analysis.

Discussion

At least one in every 100 children worldwide is affected by an intellectual disability, which not only severely limits their ability to learn, but also affects their daily lives. Mental retardation is often associated with autism-related symptoms, and studies have found that many genes are present in both disorders. The study of its pathogenesis gene provides a new idea for the diagnosis and clinical treatment of the disease, and enriches the relevant knowledge of brain development and functional expression, and ultimately provides help for individualized treatment.

In our study, 11 variants were identified, including the variant in*CREBBP, MECP2, HCFC1, ATRX, RAB39B, CLCN4, DYRK1A* and *CASK* genes. Compared with the gene-negative patients, the gene-positive patients were found to have developmental delay at an earlier age, mostly within 1 year old. Moreover, a higher incidence of complications was found in gene-positive patients, including epilepsy, ASD. One patient had severe structural abnormalities, pachygyria. And convulsions occur prior to developmental delay in some patients. There was no significant difference in intellectual development between the two groups. The genetic pattern of these 11 patients was X-linked inheritance and De Novo variation.*CREBBP* gene is widely expressed and is involved in the transcriptional co-activation of many different transcription factors, and plays a key role in embryonic development, growth control, and homeostasis by binding chromatin remodeling to transcription factor recognition. Variants in this gene cause Rubinstein-Taybi syndrome (RSTS).[10–12] Chromosomal translocations involving this gene are associated with acute myeloid leukemia. Selective splicing results in multiple transcriptional variants encoding different subtypes. In this study, we have reported and

Table 1 The Clinical Features of 21 Patients with Intellectual Developmental Disorders

Proband	Mutant Genes	Visiting Age	Clinical Phenotype	Intellectual Developmental Disorder	History of Birth	MRI
1, M	RAB39B	11 m	IDD	Mild	Normal	Normal
2, M	ATRX	22 m	IDD, EP	Mild	Premature	Normal
3, M	CLCN4	36 m	IDD, EP	Mild	Normal	Ventriculomegaly
4, M	HCFC1	41 m	IDD	Moderate	Normal	Pachygyria
5, M	HCFC1	34 m	IDD, EP	Moderate	Normal	Normal
6, M	DYRK1A	8 m	IDD, EP	Mild	Normal	Ventriculomegaly
7, F	CASK	56 m	IDD, Rett syndrome	Severe	Normal	Normal
8, F	MECP2	68 m	IDD, ASD	Moderate	Normal	Normal
9, F	MECP2	67m	IDD, ASD, EP	Moderate	Normal	Normal
10, M	MECP2	6 y9m	IDD, EP	Moderate	Normal	Normal
11, M	CREBBP	29m	IDD	Severe	Premature, Intrauterine distress	Dilated bilateral lateral ventricle and third ventricle
12, M	None	20m	IDD	Severe	Intrauterine distress	Dilated external cerebral space
13, M	None	70m	IDD	Profound	Normal	Abnormal signals from the left lateral ventricle
14, M	None	70m	IDD	Severe	Normal	Agenesis of corpus callosum
15, M	None	8m	IDD, EP	Mild	Normal	Normal
16, M	None	21m	IDD	Moderate	Normal	Normal
17, M	None	11m	IDD	Moderate	Normal	Normal
18, M	None	8m	IDD, EP	Moderate	Normal	Normal
19, M	None	13 y	IDD	Moderate	Normal	Normal
20, M	None	13 y	IDD, ASD	Moderate	Normal	Abnormal signal in the right frontal area
21, M	None	2y2m	IDD	Moderate	Normal	Normal

Notes: IDD degree: mild (55≤DQ≤75; 50≤IQ≤69); moderate (40≤DQ≤54; 35≤IQ≤49); severe (25≤DQ≤39; 20≤IQ≤34); profound (DQ<25; IQ<20).
Abbreviations: IDD, intellectual developmental disorder; EP, epilepsy; ASD, autism spectrum disorders; y, years; m, months; M, male; F, female.

analyzed a case of de novo frameshift variant in the *CREBBP* gene associated with RSTS. The variant has not been reported in the literature to date. The congenital lacrimal puncta deficiency of the right eye and the subsequent acute dacryocystitis, special facial features, broad thumbs and broad halluces, developmental delay and congenital heart disease in the patient were consistent with RSTS. The study enlarges existing knowledge of the molecular spectrum of the pathogenic *CREBBP* gene and assists the diagnosis and treatment of RSTS. The treatment of this disease is currently supported by epigenetics. It is important to follow up the growth and development.[12]

The defect in the *MECP2* gene[13] on the X chromosome (Xq28) can cause Rett syndrome, which is a complex behavioral and motor neurologic disease. Rett syndrome is a neurological disorder that often affects girls, characterized by autism-like behavior, loss of motor control, irregular breathing and bone problems. Girls with Rett syndrome

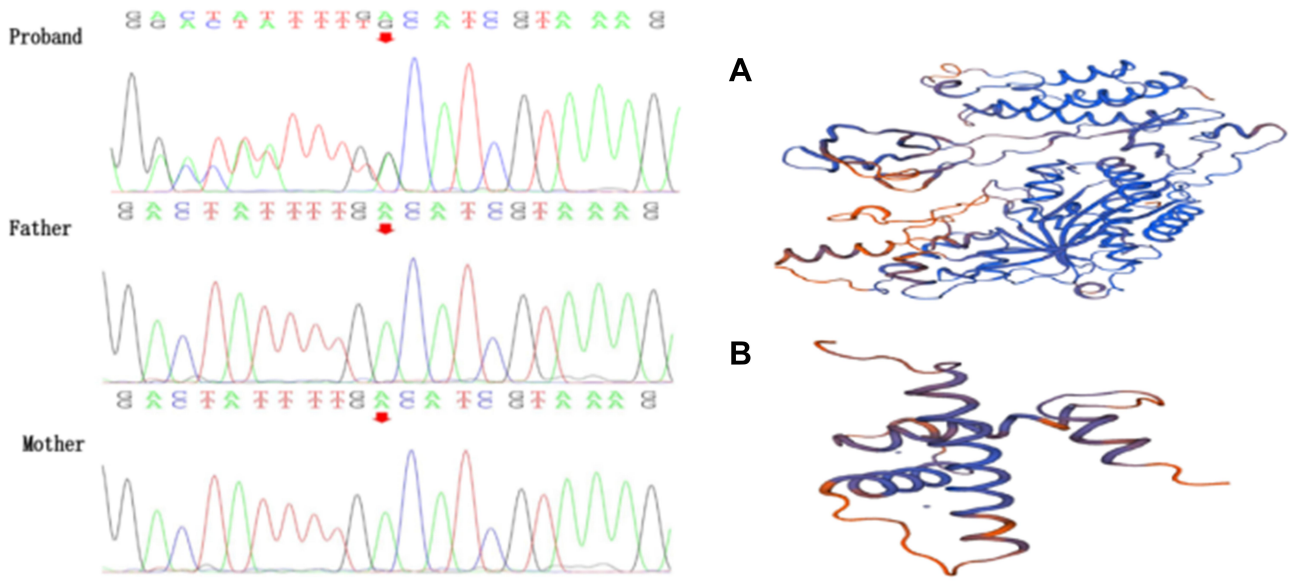

Figure 2 (Left): Chromatogram from Sanger sequencing of the CREBBP variant in the family. (Right): The protein tertiary structure of normal CREBBP protein (**A**) and mutant CREBBP protein (**B**) according the SWISS-MODEL analysis.

usually do not show symptoms until 18 months later. Rett syndrome is a sex chromosomal dominant neurological disorder that occurs in approximately 1 in 10,000 to 1 in 23,000 girls. In this study, two female patients with *MECP2* variant presented ASD phenotype, but the other patient with *CASK* variant was diagnosed Rett syndrome. *CASK*[14] is a key skeleton protein and the first protein to play a role in synaptic development. Studies have shown that *CASK* gene variant can lead to abnormal flow of calcium ions in neurons, affecting the development and plasticity of the nervous system, and is an important cause of x-linked mental retardation in humans. *HCFC1* defect[15] can cause a disorder in which intellectual function is significantly below average, associated with adaptive behavior disorders, and in which mental retardation during development is the only major symptom of non-syndromic X-associated mental retardation, whereas syndromic mental retardation is associated with physical, neurological, and/or psychiatric symptoms. In addition to developmental delays, imaging examination in proband #4 with *HCFC1* variant showed pachygyria and proband #5 developed epilepsy. These characteristics are consistent with the syndromic X-associated mental retardation. *ATRX*[16] regulates the binding of nuclear matrix and chromatin and indicates that it is involved in the interphase gene regulation and chromosome separation in mitosis. The gene variant is usually associated with X-linked alpha thalassemia/mental retardation (ATR-X) syndrome. These variants lead to different changes in DNA methylation patterns, which may provide a link between chromatin remodeling, DNA methylation, and gene expression during development. *RAB39B*[17] encodes the Rab family. Rab proteins are small GTPases involved in vesicle transport. Variants in the gene are associated with x-chromosome-related cognitive impairment. We found a *RAB39B* variant L88del in proband #1. The patient presented mild IDD. The *CLCN4* gene[18] is associated with X-linked early infantile epileptic encephalopathy (EIEE). Additionally, the *CLCN4* gene has preliminary evidence supporting a correlation with X-linked intellectual disability. The proband #3 with *CLCN4* variant suffered convulsions at 5 months of age. He had a febrile seizure followed by afebrile convulsions and developmental delay. The clinical phenotype was consistent with EIEE. *DYRK1A* kinase[19] is involved not only in neurodevelopmental processes, but also in maintaining normal brain function in adults. We found a De novo nonsense mutation in *DYRK1A*. The proband presented mild IDD and epilepsy. According to ACMG, the variant was evaluated to be pathogenic.

Conclusion

Eleven variants were identified, including the variant in *CREBBP, MECP2, HCFC1, ATRX, RAB39B, CLCN4, DYRK1A* and *CASK* genes. The function areas result of gene-positive group were not significantly different from the gene-negative group.

Table 2 The Gene Variants in 11 Children with Intellectual Developmental Disorders

Proband	Mutant Genes	Transcript	Varian Type	Nucleotide Changes	Amino Acid Changes	Position		Father	Mother	ACMG	Previous Report
1	RAB39B	NM_171998	Deletion mutation	c. 258–260delTCT	L88del	–	De novo	–	–	Pathogenic	No
2	ATRX	NM_000489	Missense mutation	c.4858A>C	S1620R	Exon18	Hemizygote	–	+	Likely pathogenic	No
3	CLCN4	NM_001830	Missense mutation	c.823G>A	V275M	Exon8	Hemizygote	N	N	Likely pathogenic	Yes
4	HCFC1	NM_005334	Missense mutation	c.4442C>T	T1481M	Exon18	Hemizygote	–	+	Likely pathogenic	No
5	HCFC1	NM_005334	Missense mutation	c.3845C>T	S1282L	Exon17	Hemizygote	–	+	Pathogenic	No
6	DYRK1A	NM_001396	Nonsense mutation	c.787 C >T	R263X,501	Exon6	De novo	–	–	Pathogenic	Yes
7	CASK	NM_003688	Splicing site mutation	c.1155+1G>A	–	IVS12	De novo	–	–	Pathogenic	No
8	MECP2	NM_004992	Nonsense mutation	c.763C>T	R255X,232	Exon4	De novo	–	–	Pathogenic	Yes
9	MECP2	NM_004992	Missense mutation	c.674C>G	P225R	Exon4	De novo	–	–	Pathogenic	Yes
10	MECP2	NM_001110792.1	Repeat mutation	c.18_23dupCGCCGC	A7_A8dup	–	Hemizygote	–	– or mosaicism	Uncertain significance	No
11	CREBBP	NM_004380	Frameshift mutations	c.3380delA	D1127AfsTer3	Exon18	De novo	–	–	Pathogenic	No

Table 3 Global Analysis of the Gesell Results

	Group	N	Average	SD	SE
Gross motor	Gene-Positive Gene-negative	10 7	51.5200 40.7143	13.96319 14.47658	4.41555 5.47163
Fine motor	Gene-Positive Gene-negative	10 7	47.7600 37.7143	14.25476 17.85790	4.50775 6.74965
Adaptability	Gene-Positive Gene-negative	10 7	48.1400 32.2857	11.84232 20.58085	3.74487 7.77883
Language	Gene-Positive Gene-negative	10 7	44.4000 37.2857	17.60808 18.24568	5.56816 6.89622
Social Competence	Gene-Positive Gene-negative	10 7	47.1700 42.0000	13.34417 18.23001	4.21980 6.89030

Table 4 Comparison of Gesell Results Between Gene Positive and Negative Group

		Levene Test			
		F	P	95% CI	
				Lower Limit	Higher Limit
Gross motor	Using Equivalent variance Not using Equivalent variance	0.211	0.653	−4.07913 −4.41387	25.69056 26.02530
Fine motor	Using Equivalent variance Not using Equivalent variance	0.410	0.532	−6.54515 −7.80348	26.63658 27.89491
Adaptability	Using Equivalent variance Not using Equivalent variance	2.302	0.150	−.87209 −3.74745	32.58066 35.45602
Language	Using Equivalent variance Not using Equivalent variance	0.024	0.879	−11.65182 −12.07116	25.88039 26.29973
Social Competence	Using Equivalent variance Not using Equivalent variance	3.243	0.092	−11.09489 −12.74571	21.43489 23.08571

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