Genes & Diseases (2022) 9, 1166–1169

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

RAPID COMMUNICATION

Comprehensive genome sequencing analyses identify novel gene mutations and copy number variations associated with infant developmental delay or intellectual disability (DD/ID)

To the Editor,

Developmental delay or intellectual disability (DD/ID) is one of the most common neurodevelopmental disabilities worldwide with high clinical and genetic heterogeneity. Its etiology remains unexplained in nearly 70% of these patients, and an accurate diagnosis still poses a challenge in clinical practice. Previous DD/ID cohort studies mostly used panel sequencing or chromosome microarray analysis (CMA), but targeted capture probes could not be updated in time, resulting in an increased risk of missed detection of genetic abnormalities. Whole-exome sequencing (WES) has been proven to result in a high diagnostic yield in patients who present with complex phenotypes. One major limitation of WES for copy number variation (CNV) analysis can be compensated by CNV-Seq technology. In this study, we conducted a retrospective study of the genetic etiology of 69 patients with unexplained DD/ID by using trio-WES and CNV-Seq. The purpose of this study was to analyze the genetic characteristics of DD/ID infants and to explore a rapid, effective, and economical genetic testing regimen.

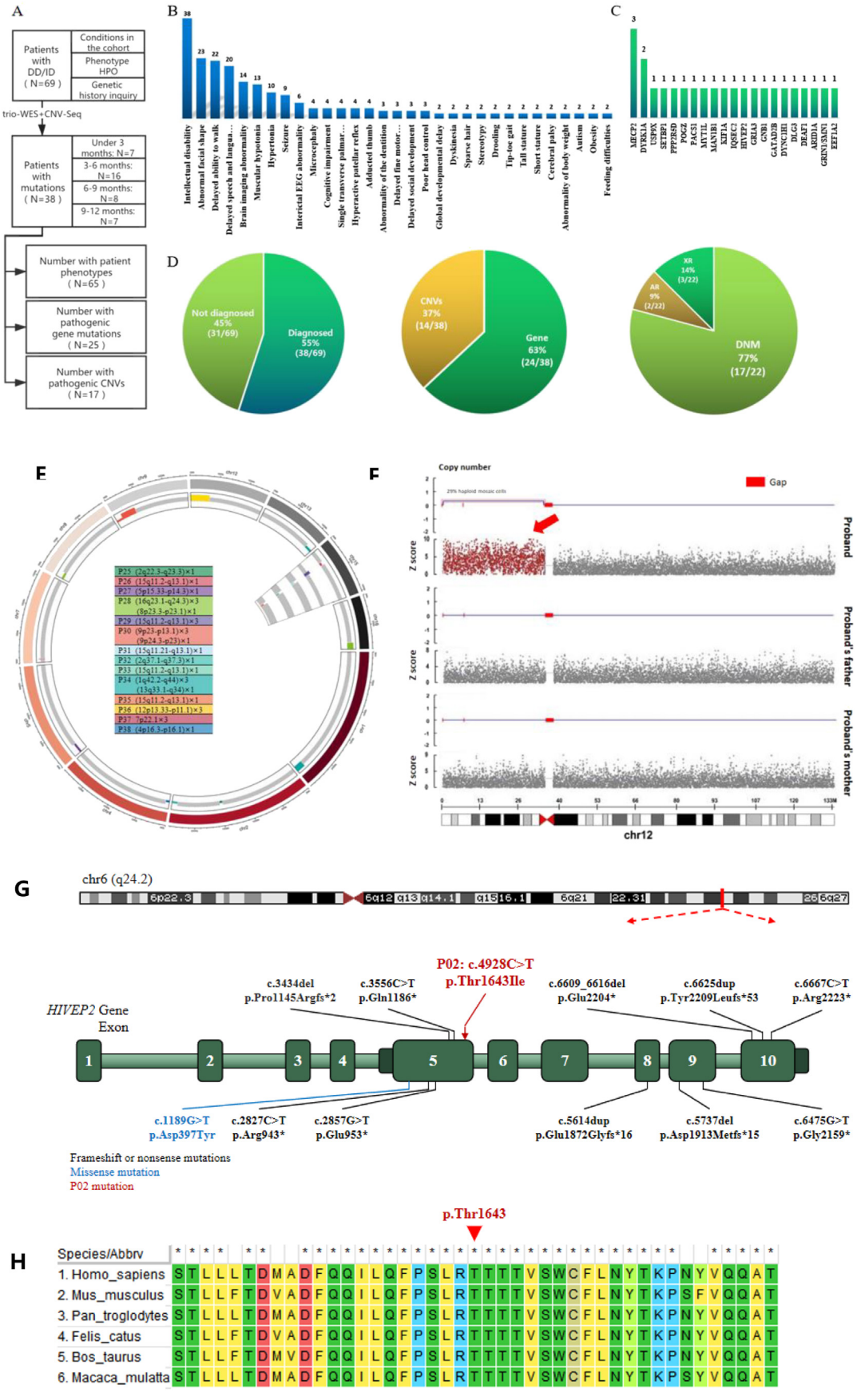
In this study, a total of 69 patients with DD/ID, with or without multiple congenital anomalies (MCA), were recruited at the Children's Hospital of Chongqing Medical University, from September 2016 to April 2020. After fully assessing the clinical phenotype, the patients and their parents were tested using trio whole-exome sequencing (trio-WES) and copy number variation sequencing (CNV-Seq). Subsequently, the clinical data and genetic variation data were statistically analyzed. An overall diagnostic yield of 55% was achieved. Among these cases, 37% were caused by CNVs, 63% by pathogenic gene mutations, and 77% by *de novo* mutations. A total of 24 cases carrying gene variants spanning 22 genes were identified, which indicated the genetic heterogeneity of DD/ID. In addition, we identified a case with low abundance somatic mosaicism (seq[grch37] dup (12) (p13.33p11.1)) (chr12:4 10001–34 822013) by CNV-Seq, and the chimeric proportion was approximately 14%. Moreover, a rare HIVEP2 missense mutation (c.4928C > T/p.T1643I, NM_006734) was identified here for the first time in China.

In this cohort, there were 35 men (51%, 35/69) and 34 women (49%, 34/69). The average onset age was 5.9 months. A total of 38 patients with underlying genetic causes were diagnosed by Trio-WES + CNV-Seq, including 21 males (55%, 21/38) and 17 females (45%, 17/38). The average onset age of these 38 cases was 6.3 months while the average age at diagnosis was 19.5 months (Fig. 1A). Most phenotypes related to neurodevelopmental disorders progress slowly. In the early stage of onset, symptoms are mild and easily neglected by their parents. The main clinical manifestations of the 69 children with DD/ID that participated in this study included mental retardation, abnormal facial shape, delayed ability to walk, delayed speech and language development, brain imaging abnormality, and muscular hypotonia, which was not significantly different from those in other similar cohorts[1] (Fig. 1B and Table S1).

Trio-WES combined with CNV-Seq was conducted to standardize and accurately diagnose for all 69 DD/ID infants, with a positive diagnosis rate of 55% (38/69). In the trio-WES group, a total of 22 genes (25 variants) were identified in the 24 cases, among which autosomal dominant inheritance accounted for 77% (17/22), all of which were *de novo* mutations (DNM) (Fig. 1C, D). The main type

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2021.11.008


A
Patients with DD/ID (N=69)
Conditions in the cohort
Phenotype HPO
Genetic history inquiry
trio-WES+CNV-Seq
Patients with mutations (N=38)
Under 3 months: N=7
3-6 months: N=16
6-9 months: N=8
9-12 months: N=7
Number with patient phenotypes (N=65)
Number with pathogenic gene mutations (N=25)
Number with pathogenic CNVs (N=17)
B
Intellectual disability
Abnormal facial shape
Delayed ability to walk
Delayed speech and langua…
Brain imaging abnormality
Muscular hypotonia
Hypertonia
Seizure
Interictal EEG abnormality
Microcephaly
Cognitive impairment
Single transverse palmar…
Hyperactive patellar reflex
Adducted thumb
Abnormality of the dentition
Delayed fine motor…
Delayed social development
Poor head control
Global developmental delay
Dyskinesia
Sparse hair
Stereotypy
Drooling
Tip-toe gait
Tall stature
Short stature
Cerebral palsy
Abnormality of body weight
Autism
Obesity
Feeding difficulties
C
MECP2
DYRK1A
USP9X
STXBP1
PPP2R5D
POGZ
FACS3
MYT1L
MAN1B1
KIF1A
IQSEC2
HIVEP2
GRIA3
CSNK2B
GATAD2B
DYNC1H1
DLG3
DEAF1
ARID1A
GRIN2B/SCN3A1
EEF1A2
D
Not diagnosed 45% (31/69)
Diagnosed 55% (38/69)
CNVs 37% (14/38)
Gene 63% (24/38)
XR 14% (3/22)
AR 9% (2/22)
DNM 77% (17/22)
E
P25 (2q22.3-q23.3)×1
P26 (15q11.2-q13.1)×1
P27 (5p15.33-p14.3)×1
P28 (16q23.1-q24.3)×3 (8p23.3-p23.1)×1
P29 (15q11.2-q13.1)×3
P30 (9p23-p13.1)×3 (9p24.3-p23)×1
P31 (15q11.21-q13.1)×1
P32 (2q37.1-q37.3)×1
P33 (15q11.2-q13.1)×1
P34 (1q42.2-q44)×3 (13q33.1-q34)×1
P35 (15q11.2-q13.1)×1
P36 (12p13.33-p11.1)×3
P37 (7p22.1)×3
P38 (4p16.3-p16.1)×1
F
Copy number
Gap
29% haploid mosaic cells
Z score
Proband
Proband's father
Proband's mother
chr12
G
chr6 (q24.2)
6p22.3
6q12
q13
q14.1
q15
16.1
6q21
22.31
26
6q27
HIVEP2 Gene
Exon
P02: c.4928C>T
p.Thr1643Ile
c.3434del
p.Pro1145Argfs*2
c.3556C>T
p.Gln1186*
c.6609_6616del
p.Glu2204*
c.6625dup
p.Tyr2209Leufs*53
c.6667C>T
p.Arg2223*
c.1189G>T
p.Asp397Tyr
c.2827C>T
p.Arg943*
c.2857G>T
p.Glu953*
c.5614dup
p.Glu1872Glyfs*16
c.5737del
p.Asp1913Metfs*15
c.6475G>T
p.Gly2159*
Frameshift or nonsense mutations
Missense mutation
P02 mutation
H
p.Thr1643
Species/Abbrv
1. Homo_sapiens STLLLTDMADFQQILQFPSLRTTTTVSWCFLNYTKPNYVQQAT
2. Mus_musculus STLLFTDVADFQQILQFPSLRTTTTVSWCFLNYTKPSFVQQAT
3. Pan_troglodytes STLLLTDMADFQQILQFPSLRTTTTVSWCFLNYTKPNYVQQAT
4. Felis_catus STLLFTDVADFQQILQFPSLRTTTTVSWCFLNYTKPNYVQQAT
5. Bos_taurus STLLFTDMVDFQQILQFPSLRTTTTVSWCFLNYTKPNYVQQAT
6. Macaca_mulatta STLLLTDMADFQQILQFPSLRTTTTVSWCFLNYTKPSYVQQAT

of DNM was missense variation (82%, 14/17) (Table S2). Trio-WES is a more effective strategy for the identification of DNM and improving the identification of candidate pathogenic genes.[2] In the CNV group, a total of 17 possible pathogenic CNVs were identified in 14 patients, and the positive diagnosis rate was approximately 20% (14/69), which was similar to that of CMA 18%.[3] High-frequency CNVs were located in 15q11.2-q13.1 (29%, 5/17, four deletions and one duplication), with an average length of 5.38 Mb. CNVs in this region have been previously associated with Prader—Willi syndrome (Fig. 1E). Loss of heterozygosity (LOH) variation accounted for about 64% (11/17) and duplication variation accounted for about 36% (6/17), with an average length of 11.56 Mb (ranging from 0.94 Mb to 34.4 Mb) (Table S3). CNV-Seq based on WGS technology has the advantages of an unbiased capture probe and balanced sequencing data.

In addition, CNV-Seq also identified a case (P36) with a 34.41 Mb repeat variation, which had low-abundance chimeric duplicate variation (about 14%) on 12p13.33-p11.1, but the patient only showed common symptoms such as DD/ID and dystonia, without other more complex clinical features (Fig. 1F). In the Decipher database (www.decipher.sanger.ac.uk/), we found several cases of chromosomal pathogenic variation in this region, among which three were chimeric duplications (ID: 267792, 295002, and 396388). The clinical features of all these cases were general retardation, mental retardation, and language retardation. The proportion of mosaicism variation varies greatly among different cell lines, therefore, there are certain challenges in the genetic counseling of CNV chimeric variants. CNV-Seq can identify chimeric variations with a lower abundance.[4] Therefore, CNV-Seq may have broader application prospects than CMA.

Moreover, trio-WES also identified a rare case (P02) carrying the *HIVEP2* mutation (c.4928C > T/p.T1643I, NM_006734), affecting the protein structure as predicted by SIFT, Polyphen2, and MutationTaster online prediction softwares, causing autosomal dominant mental retardation type 43 (MRD43). To date, only 11 cases of MRD43 have been reported, and the clinical features of MRD43 include DD/ID and delayed language development, dyskinesia, hypotonia, thin corpus callosum, and abnormal face.[5] The main phenotypes of case P02 were motor milestone backwardness, mental retardation, thumb adduction, and deformity. The facial features of the patient were micrognathia, high eyebrow arch, narrow palpebral fissure, and widely spaced eyes. Brain MRI showed delayed myelination of white matter, thin corpus callosum, and slightly widened bilateral frontotemporal extra brain space. *HIVEP2* is a transcription factor regulating various neural development pathways. The main mutation types in *HIVEP2* in the reported MRD43 cases were frameshift mutations (4/11) and nonsense mutations (6/11), and only one missense mutation (Fig. 1G, H). The novel mutation in *HIVEP2* in P02 and new phenotypes of thumb adduction identified in this study will expand the known phenotype-variation spectrum of this disease.

In conclusion, the overall diagnostic yield of Trio-WES combined with CNV-Seq for the entire cohort was approximately 55%, higher than that of CMA or WES alone. Our study provides reliable clinical phenotype and genetic data for the standardization and diagnosis of related diseases. However, this study has some limitations. First, the sample size of the cohort was small. Furthermore, there are still some blind areas in the detection range such as non-coding areas SNV, chromosome SV, and fusion variations. This study investigated the clinical and genetic characteristics of a Chinese DD/ID infant cohort, and the results showed that gene mutations and structural variations were common genetic causes of DD/ID. The combination of trio-WES and CNV-Seq can effectively improve diagnostic yields and provide important value for personalized treatment and genetic counseling of patients.

Figure 1 Comprehensive genome sequencing analyses identify novel gene mutations and copy number variations associated with infant developmental delay or intellectual disability (DD/ID). **(A)** Diagnostic process. A total of 69 patients with DD/ID were recruited. 38 patients with underlying genetic causes were diagnosed by Trio-WES + CNV-Seq, with that for seven cases (18.5%, 7/38) being 3 months of age or less, for 16 cases (42%, 16/38) 3—6 months, eight cases (21%, 8/38) 6—9 months, and seven cases (18.5%, 7/38) between 9 and 12 months. **(B)** Patients' clinical characteristics. In addition to DD/ID, the diseases phenotypes of the cohort were multiple symptoms, such as different degrees of abnormal facial shape (HP:0001999), delayed ability to walk (HP:0031936), delayed speech and language development (HP:0000750), brain imaging abnormality (HP:0410263), muscular hypotonia (HP:0001252), and hypertonia (HP:0001276), seizures (HP:0001250), and interictal EEG abnormality (HP:0025373) belonging to the high-frequency phenotype. **(C—E)** Genetic diagnosis results. Causative variants were detected in 38 probands, with a positive diagnosis rate of 55% (38/69). Among them, 24 cases (63%) were caused by gene mutations, and 14 cases (37%) were caused by CNVs. 22 genes (25 variants) were identified in the 24 cases, among which autosomal dominant inheritance accounted for 77% (17/22), all of which were *de novo* mutations (DNM), autosomal recessive inheritance accounted for about 9% (2/22), and X-linked recessive inheritance for approximately 14% (3/22). A total of 17 possible pathogenic CNVs were identified in 14 patients. High-frequency CNVs were located in 15q11.2-q13.1 (29%, 5/17, four deletions and one duplication). **(F)** Identification of low-abundance chimeric variation by CNV-Seq. A case with low abundance somatic mosaicism (seq[grch37] dup (12) (p13.33p11.1)) (chr12:4 10001—34 822013) and the chimeric proportion was approximately 14%. **(G, H)** Identification of rare HIVEP2 mutation by Trio-WES. The main mutation types in HIVEP2 in the reported MRD43 cases were frameshift mutations (4/11) and nonsense mutations (6/11), and only one missense mutation.

Conflict of interests

The authors declare that they have no conflicts of interest.

Funding

The study was authorized by the ethics committee of Children's Hospital of Chongqing Medical University.

Acknowledgements

We sincerely thank the patients and their families, as well as Chigene (Beijing) Translational Medical Research Center Co. Ltd. for providing next-generation sequencing, analyzing genetic diseases. Thank all the staff who helped us carry out the study smoothly.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2021.11.008.

References

1. Hu T, Zhang Z, Wang J, et al. Chromosomal aberrations in pediatric patients with developmental delay/intellectual disability: a single-center clinical investigation. *BioMed Res Int*. 2019;2019:9352581.
2. Iossifov I, O'Roak BJ, Sanders SJ, et al. The contribution of de novo coding mutations to autism spectrum disorder. *Nature*. 2014;515(7526):216—221.
3. Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet*. 2010;86(5):749—764.
4. Clinical Genetics Group Of Medical Genetics Branch Chinese Medical Association, Professional Committee For Prenatal Diagnosis Of Genetic Diseases Medical Genetics Branch Of Chinese Medical Association, Group Of Genetic Disease Prevention And Control Birth Defect Prevention And Control Committee Of Chinese Society Of Preventive Medicine. Expert consensus on the application of low-depth whole genome sequencing in prenatal diagnosis. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2019;36(4):293—296.
5. Srivastava S, Engels H, Schanze I, et al. Loss-of-function variants in HIVEP2 are a cause of intellectual disability. *Eur J Hum Genet*. 2016;24(4):556—561.

Yuxia Chen, Xiang Tang, Ling Liu, Qinrong Huang, Li Lin, Guoqing Liu, Nong Xiao*

Department of Children Rehabilitation, Children's Hospital of Chongqing Medical University, Ministry of Education Key Laboratory of Child Development and Disorders, National Clinical Research Center for Child Health and Disorders, China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Children's Hospital of Chongqing Medical University, Chongqing Key Laboratory of Translational Medical Research in Cognitive Development and Learning and Memory Disorders, Chongqing 400014, PR China

*Corresponding author.
E-mail address: xiaonongwl@163.com (N. Xiao)

3 June 2021
Available online 3 December 2021