The diagnostic yield of intellectual disability: Combined whole genome low-coverage sequencing and medical exome sequencing

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Abstract

Background: Intellectual disability (ID) is a heterogeneous neurodevelopmental disorder with a complex genetic underpinning in its etiology. Chromosome microarray (CMA) is recommended as the first-tier diagnostic test for ID due to high detection rate of copy number variation (CNV).

Methods: To identify an appropriate clinical detection scheme for ID in Han Chinese patients, a whole genome low-coverage sequencing was performed as the first-tier diagnostic test, followed by medical exome sequencing as the second-tier diagnostic test for those patients with negative results of CNVs.

Results: A total of 19 pathogenic CNVs in 16/95(16.84%) ID patients and 11 pathogenic single-nucleotide variations, including 7 novel mutations in 9/79(11.39%) ID patients were identified on whom no pathogenic CNVs were discovered. The detection rate of ID CNVs in multiple congenital anomalies (MCA) subgroup was significantly higher than ID with autism spectrum disorder and the other IDs subgroup. Also single-nucleotide variations showed a higher occurrence rate in the other IDs subgroup.

Conclusions: There were differences in the diagnostic yields of different variation types among the three ID subgroups. Our findings provided a new perspective on appropriate clinical detection scheme for different ID subgroups based on the statistical results of different ID subgroups. The application of whole genome low-coverage sequencing as the first-tier diagnostic test for ID with MCA subgroup and medical exome sequencing as the first-tier diagnostic test for other IDs subgroup were considered as an efficient clinical detection schemes.

Background

Intellectual disability (ID) is characterized by impairment of cognitive and social adjustment due to brain damage or incomplete development, which accounted for a prevalence of about 1–3% in worldwide^{1–4}. ID is a complicated neurodevelopmental disorder that might be affected by a series of heterogeneous factors such as environmental, genetic, idiopathic, neonatal sequelae and other diseases². Many studies have been conducted to reveal the genetic etiology of ID and there are more than 600 genes and 130 rare copy number variations (CNVs) involved in cause of ID^{5–9}. However, the causes in up to 50% cases cannot be found due to complex etiological factor and high genetic heterogeneity^{7,10–13}.

The well-known variant types of the causes of ID included chromosome number or structure abnormality, genome-wide microdeletion or microduplication and single gene defect. Chromosome microarray (CMA) has been recommended as the first-tier diagnostic test for ID and so, CNVs may have the highest positive rate with regard to ID^{14,15}. Most of the previous studies conducted on genetic etiology of ID were based on CMA^{16–18}. According to a previous study, chromosome abnormality and genome-wide microdeletion or microduplication accounted for 10%-20% of ID^{3,19}. Although CMA has been recommended as the first-tier diagnostic test for ID, it is still limited due to insufficiency in detecting single gene defects.

With the rapid development and wide-use of next generation sequencing in the clinical diagnostic field, the novel technologies conducted genetic research on ID with a rapid approach to identify more causative CNVs and genes^{7,20,21}. Previous studies have shown that significant parts involved in ID patients, with negative results after CMA, and included a single gene defect. Single gene defect may account for about 10% of ID⁷. As more and more novel or candidate genes were discovered, it could be even higher. A number of novel ID candidate genes, such as *ASH1L*, *MBOAT7* and *TRIO*, were identified by using next generation sequencing in patients who had negative results after CMA^{22–24}.

Most of the genetic studies on intellectual disabilities were based on European populations. The ID genetic research studies on Chinese population were less. And majority of these studies focused on CNVs through CMA¹⁸. Investigating the distribution of CNVs, proportion of single gene defects, and evaluation of effects of different diagnostic platforms in Chinese ID populations provided the proof for selecting appropriate method of clinical genetic diagnosis in Chinese ID

populations. We herein performed a whole genome low-coverage sequencing as the first-tier diagnostic test in 95 Chinese ID patients and medical exome sequencing was applied as the second-tier diagnostic test for those patients with negative results of CNVs to search for any additional single gene defects. Pathogenic CNVs and single gene mutations were identified by both clinical phenotype relevance and genetic interpretation. We compared the detection rates of CNVs and single gene defects in our study with the previous study. Furthermore, we subdivided ID patients into three different subgroups, ID with multiple congenital anomalies (MCA), ID with autism spectrum disorders (ASD) and other IDs. Compared with the detection rates of CNVs and single gene defects among the three subgroups provides a new vision on the choice of appropriate clinical diagnostic test method in different ID patients.

Methods

Subjects and controls

The patients were recruited from the Department of Neurology, Affiliated Children's Hospital of the Capital Institute of Pediatrics between 2016.01 and 2018.12. Written informed consent was obtained from all parents (Ethical No. SHERLL2013046). All enrolled 95 patients (30 females and 65 males) had clinical manifestations of ID. The patients were subdivided into three subgroups according to whether they had MCA or ASD except ID (ID with MCA, 52 patients; ID with ASD, 14 patients; and other IDs, 29 patients).

Whole genome low-coverage sequencing

Genomic DNA was extracted from the peripheral blood sample using QIAamp DNA Mini Kit (QIAGEN). The DNA was quantified by using Nanodrop 2000 (Thermal Fisher Scientific, DE). The DNA was sheared to size by approximately 300 bp with a Covaris S2 sonicator according to the Illumina TruSeq DNA protocol. The fragments were then end-repaired and A-tailed in preparation for ligation to adapters. The ligation product was amplified by PCR by using universal primers. The enriched libraries were sequenced on an Illumina HiSeq 2000 sequencer (Illumina, San Diego, CA, USA) for paired-end reads of 150 bp. High-quality paired-end reads were aligned to the NCBI human reference genome hg19 by using Short Oligonucleotide Analysis Package (SOAP) aligner software (SOAP2.21; soap.genomics.org.cn/soapsnp.html)²⁵. CNV detection was performed according to a three-step method reported previously^{26,27}.

The CNV Interpretation

To evaluate the pathogenicity of CNVs, the criteria of ACMG guidelines for CNVs in 2011 were mainly referred²⁸. A CNV was evaluated as a pathogenic CNV when it complies with one of the following criteria: when 1) it overlaps with at least 50% of the critical region of known genomic disorders; 2) it contains known ID disease-causing genes and the variation of dosage effect conform to the genetic pattern; and 3) the size of the CNV is larger than 1 Mb and the region has been recorded in patients with ID in DECIPHER. CNV was evaluated as an uncertain clinical significant CNV when it was documented in < 0.1% of the population and did not meet the criteria of pathogenic CNVs.

Medical exome sequencing

Genomic DNA was extracted from the peripheral blood by using QIAamp DNA Mini Kit (QIAGEN). The DNA was quantified with Nanodrop 2000 (Thermal Fisher Scientific, DE). A minimum of 3 ug DNA was used for the indexed Illumina libraries according to the manufacturer's protocol. DNA fragments with sizes ranging from 350 bp to 450 bp and those including the adapter sequences were selected for DNA libraries. Next, over 4000 genes associated with monogenic disorders were selected by a gene capture strategy by using a custom enrichment kit (IDT, Coralville, Iowa, USA). The enriched libraries were sequenced on an Illumina HiSeq XTen sequencer (Illumina, San Diego, CA, USA) for paired-end reads of 150 bp. Following sequencing, the raw image files were processed by using Bcl2Fastq software (Bcl2Fastq 2.18.0.12, Illumina, Inc.) for base calling and raw data generation. Low-quality variations were filtered out by a quality score of ≥ 20 . SOAP aligner software (SOAP2.21; soap.genomics.org.cn/soapsnp.html) was then used to align the clean reads with the reference human genome

(hg19). Polymerase chain reaction (PCR) duplicates were removed by using the Picard program. Subsequently, single nucleotide polymorphisms (SNPs) were determined by using the SOAPsnp program, reads were realigned by using Burrows-Wheeler Aligner software 0.7.15, and the insertions and deletions (InDels) were detected by using Genome Analysis Toolkit software 3.7. The identified SNPs and InDels were annotated by using the Exome-assistant program. The pathogenicity of SNPs and InDels was evaluated according to ACMG guidelines.

Results

Rare copy number variations

To explore whether the ID was caused by rare CNVs, a whole genome low-coverage sequencing was used as the first-tier diagnostic test in 95 patients diagnosed with ID. As many point variations were single nucleotide polymorphism, several genome-wide CNVs showed polymorphism²⁹. Therefore, the rare CNVs with population frequency < 0.1% were mainly focused. A total of 312 rare CNVs were identified in 95 patients including 175 gains and 137 losses. The size of the rare CNVs in patients ranged from 102 Kb to 155 Mb. According to the criteria of evaluation of the pathogenicity of rare CNVs in the Methods section, 19 pathogenic CNVs were identified in 16 patients (Fig. 1a, Table 1). Fourteen CNVs (8 losses and 6 gains) were associated with known genomic disorders including 2q31.1 microdeletion syndrome, 7q31 microdeletion syndrome, 7p duplication syndrome, 9p duplication syndrome, 10q deletion syndrome, Jacobsen syndrome, Prader-Willi and Angelman syndrome, Renal cysts and diabetes (RCAD), 17p11.2 duplication syndrome, Smith-Mageni syndrome, 2q37 deletion syndrome, and Klinefelter syndrome (Table 1, Patient 1, 3–16). We also identified a 4.43 Mb duplication at 5q35 containing the dosage sensitivity gene NSD1 that contributed to Sotos syndrome (Table 1, Patient 2). Four CNVs were larger than 1 Mb and the region has been recorded in patients with ID in DECIPHER included a 2.92 Mb deletion at 2q24.1, a 6.36 Mb duplication at 19q13.42-13.43, a 15.04 Mb duplication at 20p12.1-p13 and a 11.66 Mb deletion at 9p23-24.3 (Table 1, Patient 1, 12, 13, 14).

Two concurrent pathogenic CNVs in one patient

Cytogenetic imbalances are the most frequently identified causes of ID/MCA. The diagnostic rate of submicroscopic terminal rearrangements was about 6%, with a range of 2–29% due to different resolutions of techniques, inclusion criteria and sample sizes³⁰. Meanwhile, a frequency of two or more chromosomal aberrations was estimated to be 2–4% by previous studies³¹. Two concurrent pathogenic CNVs were identified by whole genome low-coverage sequencing in 4 of the 16 CNV-positive patients (Table 1, Patient 1, 12, 13, 14), accounting for 25% in CNV-positive patients and 4.2% in the total 95 patients, respectively. The rate was generally consistent with that reported by a previous study based on a large-scale cohort of ID. We identified a 2.06 Mb deletion in 2q31.1 region in 1 patient with a concurrent 2.92 Mb deletion in 2q24.1 region. Patient 14 has a 11.66 Mb deletion in the 7p22.3-p31.2 region and a concurrent 14 Mb duplication in 9p23-24.3 region. Two patients, 12 and 13, carried a deletion in the 2q37 region, with a concurrent 6.36 Mb duplication of the distal breakpoint at 19q13.41 and 15.04 Mb duplication of the distal breakpoint at 20p12.1, respectively (Fig. 3a). Both large deletion and duplication in subtelomeric region were detected in patients 11, 12, and 13, suggesting it to be probably caused by subtelomeric rearrangement. These findings highlighted the importance of screening apparently “balanced” subtelomeric rearrangements inherited from a phenotypically normal parent in patients with ID.

Variable clinical phenotypes of CNVs

In patient 9, a 1.49 Mb deletion was found at 17q12, and was known to be associated with renal cysts and diabetes (RCAD), (Fig. 3d). The most consistent clinical feature of RCAD is the presence of renal cysts and most of the affected subjects also had early-onset diabetes. However, Patient 9 in our study showed no signs of renal abnormalities and diabetes. Patient 9 showed clinical manifestations of asophia, and brain dysplasia was revealed by MRI instead. Interestingly, the recurrent 17q12 deletion has also been documented with diverse range of phenotypes associated with neurodevelopment, such as autism spectrum disorder and attention-deficit hyperactivity disorder^{32,33}, except RCAD. The 1.49 Mb deletion at 17q12

contains 14 protein-coding genes in addition to HNF1B, which was considered as the etiology of RCAD^{34,35}. This suggested that there might be other protein-coding genes at 17q12 that contributed for the neurodevelopmental disorder, such as *LHX1* and *ACACA*, and were inferred as the genes involved in neurodevelopmental syndrome^{36,37}.

Both patients 12 and 13 carried a deletion in 2q37 region and presented facial dysmorphism and global developmental delay. Some other different clinical symptoms were still observed in 2 patients. In addition, a neonatal hypoglycemia was observed in patient 12 and a reproductive system abnormality was observed in patient 13. The variable clinical phenotypes between patients 12 and 13 were probably due to different deletion sizes (7.92 Mb vs 2.3 Mb) at 2q37 (Fig. 3c). The other explanation for these was the additional duplication regions identified in 2 patients, respectively, as both 19q13.42q13.43 and 20p12.1-13 duplication (Fig. 3a, 3b) have been reported with ID and craniofacial dysmorphisms by few case reports^{38,39}.

Single-nucleotide variations

The medical exome sequencing of 79 patients showed negative results by whole genome low-coverage sequencing. Then our analysis on the genes that are previously associated with ID or related neurodevelopmental disorders was focused. We identified 11 predicted pathogenic variants in 9/79 patients (Table 2). These variants included de novo (n = 5), compounded heterozygous (n = 2), homozygous (n = 1) and hemizygous variants (n = 2). No genes were found to be mutated in two families, suggesting a low incidence of hot spots underlying in ID in Han Chinese population. Compared with the phenotypes of 11 variants in genes that are known to cause ID, most of the patients reported similar phenotypic entity. However, some deviations do occur, which might result due to the clinical diversity or by the age of onset. The clinical features of patient 25 included learning disability, hyperactivity, epilepsy, congenital heart disease and moderate ID. Medical exome sequencing showed a novel homozygous variant in *AP4M1*, causing a replacement of serine by phenylalanine at position 9 of the protein. *AP4M1* was reported to be the genetic cause of Spastic paraplegia 50, which is characterized by severe intellectual disability, spasticity, developmental delay, facial features and microcephaly on postnatal onset³⁹. Patient 25 did not present with spasticity or dystonia but with congenital heart disease instead, and has not been reported in previous patients with *AP4M1* mutations. Our findings might expand *AP4M1* phenotypes to cardiac abnormality.

Novel mutations identified by medical exome sequencing

We identified 7 novel pathogenic or likely pathogenic single-nucleotide variations in 6/9 ID patients, which included *HPRT1* c.419delG, *AARS2* c.806G>A/c.374T>C, *KIF1A* c.1262A>C, *STXBP1* c.536T>G, *B4GALT7* c.319G>C and *AP4M1* c.26C>T (Table 2, Patients 17, 18, 21, 23, 24, and 25). In patient 17, a hemizygous 1-bp deletion (419delG) that caused a frameshift was found at exon 6. The mutation resulted in premature termination of translation of *HPRT* mRNA (p.Gly140Alafs*26), which was not reported previously but a pathogenic missense amino acid change occurred at the same position⁴⁰. A novel compounded heterozygous mutation, c.806G>A and c.374T>C, was revealed in *AARS2* gene in patient 18 who presented with psychomotor retardation, hypotonia, cerebellar atrophy and white matter abnormal signal. Both the missense mutations substituted highly conserved amino acid residues in aminoacylation domain, and were predicted to be damaging by SIFT, Polyphen2 and Mutation Taster. Two novel de novo mutations, *KIF1A* c.1262A>C and *STXBP1* c.536T>G were identified in patients 21 and 23, respectively. In patient 24, a novel missense mutation c.319G>C caused heterozygous mutation of a novel compound with a reported pathogenic missense mutation at p.Leu205Pro in the *B4GALT7* gene. In addition, a homozygous mutation was also identified in *AP4M1*, which is a disease-caused gene of Spastic paraplegia 50, encoding for the m subunit of adaptor protein complex-4 in patient 25³⁹.

Diagnostic yield and variation types in different ID subgroups

The diagnostic yields informed in previous studies of large-scale cohort of IDs were mostly acquired by a mixed ID group that encompassed other phenotypes including MCA and ASD. Previous studies reported an increased diagnostic yield of CNVs in ID patients with MCA^{41,42}. To investigate whether the diagnostic yields showed differences among different ID

subgroups, ID patients were subdivided into 3 different subgroups, ID with MCA, ID with ASD and other IDs. Diagnostic yield varied according to the ID subgroups. The highest diagnostic rate was observed in other ID subgroup (34.48%), followed by ID with MCA (26.92%). The lowest diagnostic rate was observed in ID with ASD subgroup (7.14%), (Fig. 2b). Except diagnostic yield, the variation types were also varied by ID subgroups. In ID, the MCA CNVs accounted for 23.07% and single gene defects accounted for 3.85% (Fig. 2b). In other IDs, CNVs accounted for 10.34% and single gene defects accounted for 24.14% (Fig. 2b). The rate of ID CNVs in MCA subgroup was significantly higher than the other two subgroups. Likewise, single gene defects contributed to a larger proportion in other ID subgroup. The varied diagnostic yields and variation types suggested that the clinical detection scheme of ID might be changed along with different ID subgroups that patients belong to. These result provided us a new perspective on the appropriate clinical detection scheme for different ID subgroups.

Discussion

A total of 19 pathogenic CNVs in 16.84% (16/95) of Han Chinese ID patients were found by whole genome low-coverage sequencing. This rate was mainly consistent with a previous study based on European and Chinese populations by CMA⁴³⁻⁴⁵. Although there was a subtle difference, which might be due to the differences in technology platforms or the differences in the criteria applied to evaluate the pathogenic level of CNVs. These results validated whole genome low-coverage sequencing as an alternative effective diagnostic method for genome-wide CNV detection in routine clinical application⁴⁶.

Due to relatively little realization of the CNVs when compared with single-nucleotide variations, the database of pathogenic CNVs and well-known genome-wide disorders still remained poor. Therefore, there were several CNVs judged as variants with uncertain significance. Among CNVs that are associated with well-known genome-wide disorders in patients, a 1.49 Mb deletion at 17p11.2 locus was reported by RCAD in a patient characterized by ID, cerebral dysplasia, asophia, big head circumference and slightly lower limb muscle tone as the patients presented with ID or global developmental delay, which was documented by a previous study that focused on neurodevelopmental disorders. Although the two patients who carried 2q37 deletion had ID and facial dysmorphism, but also showed some varied phenotypes when compared to each other. The diversity in phenotypes might be explained by different boundaries of deletion regions encompassing different genes among different patients or additional modified variations in other areas such as epigenetics^{35, 47, 48}. This indicated that the study of pathogenic mechanisms of CNVs were more complicated than SNVs. The genetic contributors were yet to be revealed for many pathogenic CNVs and even for some well-known disorders such as 16p11.2 microdeletion syndrome.

In addition to the CNVs, we applied medical exome sequencing to screen single-nucleotide variations in patients where no pathogenic CNVs were detected. Diagnostic yield showed improvement as the resolution of cytogenetic testing in patients with developmental disabilities has been evolved. We identified 11 pathogenic mutations among 9 genes in 9 unrelated patients. No two patients carried the same disease-causing gene. This indicated a strong genetic heterogeneity and no hot spot genes for ID in Han Chinese population. The disease type caused by the genes varied from syndromic and non-syndromic ID to epileptic encephalopathy, leukoencephalopathy and spastic paraplegia, showing a strong clinical heterogeneity. This might explain the low detection rate (~ 10%) of whole exome sequencing for ID patients as previous study⁴⁹.

The patients with multiple congenital anomalies may have a higher detection rate of CNVs than other ID patients^{41, 42}. Therefore, the ID patients were subdivided into 3 subgroups, including ID with MCA, ID with ASD and other IDs. Diagnostic yield and variation types were different in different ID subgroups. ID with MCA subgroup showed a higher detection rate of CNVs. ID with ASD showed the lowest detection rate in both CNVs and single-nucleotide variations. More single-nucleotide variations were identified in other ID subgroups. This suggested that the proportion of variation types were significantly different in different subgroups.

Seventy (73.69%) patients with ID have still not received a molecular diagnosis in our study. This was consistent with the results conducted by previous studies where a majority of ID cases remain undiagnosed after genetic detection due to different technology platforms such as microarray, ID panel and whole exome sequencing^{7, 10-13}. Such a high negative rate

might be caused by the limitation in resolution of CNVs, the detection area of sequencing and the sensitivity of low frequency mosaic mutation⁶. The resolution of CNVs through whole genome low-coverage sequencing is 100 kb, which meant that the smaller CNVs cannot be detected such as single-exon and intra-exonic deletions. Medical exome sequencing was applied due to its cost-effective detection scheme for single-nucleotide variations. Meanwhile, the novel genes might be omitted and the mutations outside the coding regions remain to be further explored. Recent studies have revealed that the low frequency mosaic mutation might play a role partly in neurological and neuromuscular disorders^{6, 50}.

Conclusions

Although there were many genetic studies that focused on uncovering the etiology of ID, the information of ID in Han Chinese population was still little. Most of the previous studies were based on CMA. We herein performed whole genome low-coverage sequencing as the first-tier diagnostic test and medical exome sequencing as the second-tier diagnostic test for those patients with negative results of CNVs. The detection rates of CNVs and SNVs were consistent with those of the previous studies at home and abroad. This detected scheme was considered appropriate and cost-effective in terms of the consequences. The significantly different proportions of variation types prompted us to select the appropriate detection scheme for different ID subgroups based on the results of this study. However, the sample size of this study is small, and the conclusion might be influenced by sample bias. This study should be replicated with a larger study to eliminate the influence of patient's source selection. As a result, a larger and multicenter study should be conducted. Our study provided a new perspective regarding the study of clinical molecular diagnosis for ID patients in Han Chinese population.

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the ethical committee of Affiliated Children's Hospital of Capital Institute of Pediatrics, China and Capital Characteristic Clinical Application Research (Z131107002213159). Informed consent was obtained from the patients, and for those patients under 18 years old, consent was obtained from the parents on their behalf.

Availability of data and materials

To ensure patient confidentiality, data containing potentially identifiable information was not shared. All data generated or analysed during this study, without identifiable information, is available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing financial interests.

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Authors' contributions

Jun Wang and Yan Wang performed the analysis, prepared the figures and tables, wrote and reviewed the manuscript. WangYang Chen and Min Sheng performed the whole genome low-coverage sequencing and medical exome sequencing. Liwen Wang and Yan Wang recruited the patients and collected clinical information. All authors have read and approved the manuscript.

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Tables

Table 1. Pathogenic copy number variations identified by whole genome low-coverage sequencing.

Patient	CNV variation	Region size	Known genomic disease	Patient documented in Decipher Database
1	del(2q24.1) (155.48Mb-158.4Mb)*1 del(2q31.1-q31.2) (176.16Mb-178.22Mb)*1	2.92Mb 2.06Mb	2q31.1 microdeletion syndrome	2:154961717-158712890*1,Pathogenic,3.75Mb; 2:156830779-159106872*1,Unknown,2.28Mb; Delayed speech and language development, Intellectual disability, Macrocephaly, Tall stature; 2:155796265-157725469*1,Unknown,1.93Mb; Global developmental delay, Intellectual disability, severe
2	Dup(5q35.2-q35.3) (175.74Mb-180.08Mb)*3	4.43Mb	Sotos syndrome(<i>NDS1</i>)	5:175714974-180696832*3,Pathogenic,4.98Mb; Brachycephaly, Hypertelorism, Microcephaly, Short stature, Unilateral ptosis; 5:175207164-180694002*3,Unknown,5.49 Mb
3	del(7q31.1-q31.33) (113.9Mb-125.52Mb)*1	11.62Mb	7q31 microdeletion syndrome (? <i>FOXP2</i> , <i>DOCK4</i> , <i>MET</i> , and <i>WNT2</i>)	7:114921919-126025662*1,Unknown,11.10 Mb; 7:114236695-127881806*1,Unknown,13.65 Mb; Delayed speech and language development, Intellectual disability, Muscular hypotonia; 7:112510560-121723279*1,Unknown,9.21 Mb; Aplasia/Hypoplasia of the thymus, Autism(Other) ; Delayed speech and language development, Intellectual disability, Seizures(46XX) 7:112137064-119186429*1,Pathogenic,7.05 Mb; Abnormal facial shape, Moderate global developmental delay
4	dup(7p14.3-p22.3)(0.1Mb-30.82Mb)*3	30.82Mb	7p duplicationsyndrome	7:10239-25112979*3,Unknown,25.10 Mb: Absent speech, Constipation, Global developmental delay, Intellectual disability, severe, Long fingers, Low-set ears, Micrognathia, Muscular hypotonia, Narrow mouth, Pancreatitis, Thoracolumbar scoliosis 7:503373-11090297*3,Pathogenic,10.59 Mb; Autism, Broad forehead, Diastema, Generalized joint laxity, Global developmental delay, Micrognathia, Self-injurious behavior, Wide nose 7:109626-16317319*3,Unknown,16.21 Mb; Abnormality of prenatal development or birth, Cleft palate, Depressed nasal bridge, Low-set ears, Micrognathia
5	dup(9p22.2-p23) (10.06Mb-16.78Mb)*3	6.72Mb	9p duplication syndrome	9:9910369-19437090*3,Likely pathogenic, 9.53 Mb; Aplasia/Hypoplasia of the distal phalanges of the hand, Aplasia/Hypoplasia of the distal phalanges of the toes, Aplasia/Hypoplasia of the middle phalanges of the hand, Aplasia/Hypoplasia of the middle phalanges of the toes, Cupped ear, Diastasis recti, Epicanthus, Glabellar hemangioma, Hemangioma, Hypertelorism, Low-set ears, Nail dystrophy, Relative macrocephaly; 9:8266233-16527801*3,Unknown, 8.26 Mb; Global developmental delay
6	del(10q26.13-q26.3) (126.62Mb-135.52Mb) *1	8.9Mb	10q deletion syndrome	10:126198009-135430043*1,Pathogenic,9.23Mb (Imbalance arising from a balanced parental rearrangement) ; 10:127120633-135427143*1,Likely pathogenic,8.31Mb; Congenital strabismus, Constipation, Generalized hypotonia, Language

				<p>impairment, Microcephaly, Moderate expressive language delay, Sleep-wake cycle disturbance, Temperature instability;</p> <p>10:125632306-135434148*1,Unknown,9.80 Mb; Functional abnormality of the bladder, Intellectual disability, moderate, Patent urachus, Strabismus(Imbalance arising from a balanced parental rearrangement)</p>
7	del(11q24-q25) (124.55Mb-134.94Mb)*1	10.39Mb	Jacobsen syndrome	11:124205261-134868378*1,Pathogenic,10.66 Mb; Abnormal platelet count, Epicanthus, Global developmental delay, Hypertelorism, Low-set ears, Short nose, Short stature, Smooth philtrum, Thin upper lip vermilion, Thrombocytopenia
8	del(15q11.2-q13.2) (23.62Mb-30.38Mb)*1	6.76Mb	Prader-Willi and Angelman syndrome	<p>15:23619912-28438266*1,4.82 Mb; EEG abnormality, Intellectual disability, Microcephaly, Seizures, Truncal ataxia;</p> <p>(15:23619912-28438266*1,4.82 Mb; Feeding difficulties in infancy, Hypogonadism, Intellectual disability, Muscular hypotonia, Truncal obesity;</p> <p>15:23699760-30322138*1,Pathogenic,6.62 Mb; Absent speech, Global developmental delay, Seizures</p>
9	chr17:34918482-36408851*1	1.49Mb	Renal cysts and diabetes (RCAD)	<p>17:34815072-36215917*1,1.40 Mb; Abnormality of the liver, Diabetes mellitus, Multiple renal cysts;</p> <p>17:34911952-36510799*1,Pathogenic,1.60 Mb; Fetal choroid plexus cysts, Multicystic kidney dysplasia</p>
10	dup(17p11.2-p12) (14.76Mb-19.48Mb)*3	4.72Mb	17p11.2 duplication syndrome/CMT1A	17:16773072-20222149*3,3.45 Mb; Autism, Hyperactivity, Short attention span, Short stature; 17:14097915-15470903*3,1.37 Mb; Abnormality of the motor neurons, Decreased motor nerve conduction velocity, Hypertrophic nerve changes, Impaired pain sensation, Impaired proprioception, Impaired temperature sensation, Impaired vibratory sensation, Pes cavus
11	del(17p11.2) (16.6Mb-20.5Mb)*1	3.9Mb	Smith-Mageni syndrome	17:16590776-20463301*1,Pathogenic,3.87Mb;Brachydactyly, Severe global developmental delay, Specific learning disability
12	del(2q37.2-37.3) (235.16Mb-243.08Mb) *1 dup(19q13.42-q13.43) (52.76Mb-59.12Mb)*3	7.92 Mb 6.36 Mb	2q37 deletion syndrome	<p>2:235875302-243041364*1,Pathogenic,7.17 Mb; Cognitive impairment, Horseshoe kidney, Postnatal microcephaly, Seizures, Underdeveloped nasal alae;</p> <p>19:51294464-56379713*3,Likely pathogenic,5.09 Mb; Intellectual disability, Seizures, Short stature, Specific learning disability;</p> <p>19:53181823-59095418*3,Unknown,5.91 Mb; Hypodysplasia of the corpus callosum, Mild global developmental delay, Noncommunicating hydrocephalus;</p> <p>19:53569329-59052715*3,Unknown,5.48 Mb; Global developmental delay</p>
13	del(2q37.3) (240.70Mb-243Mb)*1	2.30Mb 15.04Mb	2q37 deletion syndrome	2:235875302-243041364*1,Pathogenic,7.17 Mb; Cognitive impairment, Horseshoe kidney, Postnatal microcephaly, Seizures, Underdeveloped nasal alae;

	dup(20p12.1-p13)(0.1Mb-15.14Mb)*3			20:121521-9691972*3,Pathogenic,9.57 Mb, Intellectual disability, mild, Tall stature; 20:121521-3783829*3,Pathogenic Partial,3.66 Mb; Global developmental delay; 20:207270-5862333*3,Unknown,5.66 Mb; Abnormality of the nervous system, Tremor
14	del(9p24.3-p23)(1Mb-12.66Mb)*1 dup(7p22.3-p21.2)(1Mb-15Mb)*3	11.66Mb 14Mb	9p deletion syndrome 7p duplication syndrome	9:271257-12907826*1,Pathogenic,12.64Mb;Abnormality of the eye, Absent speech, Bilateral cryptorchidism, Bilateral talipes equinovarus, Cognitive impairment, Downslanted palpebral fissures, Generalized hypotonia, Hypoplasia of the corpus callosum, Micrognathia, Seizures, Synophrys, Ventricular septal defect 7:109626-16317319*3,Unknown,13.64Mb;Deep plantar creases, Frontal bossing, Hydrocephalus, Hypertelorism, Intellectual disability, Midface retrusion, Stenosis of the external auditory canal
15	47,XXY	X chromosome	Klinefelter syndrome	-
16	47,XXY	X chromosome	Klinefelter syndrome	-

Table 2 Pathogenic gene mutations identified by medical exome sequencing.

Patient	Gene	Nucleotide change	Amino acid change	Het/Hom	Related disease	origin	Literature report
17	HPRT1	c.419delG	p.Gly140Alafs*26	hemi	Lesch-Nyhan syndrome	maternal	Novel
18	AARS2	c.806 G>A	p.Gly269Asp	het	Leukoencephalopathy, progressive, with ovarian failure	Paternal	Novel
		c.374T>C	p.Leu125Pro	het		Maternal	Novel
19	EEF1A2	c.796C>T	p.Arg266Trp	het	Intellectual disability, autosomal dominant 38	de novo	Helbig,et al, 2016
20	TCF4	c.1153C>T	p.Arg385Ter	het	Pitt-Hopkins syndrome	de novo	Zweier,et al, 2007
21	KIF1A	c.1262A>C	p.His421Pro	het	Intellectual disability, autosomal dominant 9	de novo	Novel
22	CACNA1A	c.4991G>A	p.Arg1664Gln	het	Migraine, familial hemiplegic, 1	de novo	Tonelli,et al, 2006
23	STXBP1	c.536T>G	p.Leu179Arg	het	Epileptic encephalopathy, early infantile, 4	de novo	Novel
24	B4GALT7	c.319G>C	p.Glu107Gln	het	Ehlers-Danlos syndrome, spondylodysplastic type, 1	Paternal	Novel
		c.614T>C	p.Leu205Pro	het		Maternal	Okajima,et al, 1999
25	AP4M1	c.26C>T	p.Ser9Phe	hom	Spastic paraplegia 50	Paternal Maternal	Novel

Figures

Figure 1

Status of molecular diagnosis after CNV-seq and MES of 95 patients with intellectual disability. a) The detection rates of CNVs and SNVs. b) The proportion of different CNVs types. c) The inheritance patterns of diseases detected by MES.

a

b

	ID with MCA		ID with ASD		Other ID	
	Number	Percentage	Number	Percentage	Number	Percentage
Pathogenic CNV	12	23.07%	1	7.14%	3	10.34%
Pathogenic SNV	2	3.85%	0	0%	7	24.14%
Diagnostic rate	14	26.92%	1	7.14%	10	34.48%
VUS CNV	11	21.15%	2	14.29%	12	41.38%
Negative	27	51.93%	11	78.57	7	24.14%
Total sample number	52	100%	14	100%	29	100%

Figure 2

Diagnostic yield in ID cohort (n=95) by subgroup distribution through whole genome low-coverage sequencing and medical exome sequencing.

Figure 3

Map of CNVs associated with varying phenotypes of known genomic disorders. a) Two concurrent pathogenic CNVs, 2q37.1-q37.3 deletion and 19q13.42-q13.43 duplication, in patient 12. b) Two concurrent pathogenic CNVs, 2q37.3 deletion and 20p12.1-p13 duplication, in patient 13. c) Map of 2q37 deletions with included genes in 2 patients. and d) Map of 17q12 deletions with included genes in patient 9.