

# Clinical and genetic characteristics and prenatal diagnosis of patients presented GDD/ID with rare monogenic causes

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## Research

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# Abstract

## Background:

Global developmental delay/intellectual disability (GDD/ID), used to be named as mental retardation (MR), is one of the most common phenotypes in neurogenetic diseases. In this study, we described the diagnostic courses, clinical and genetic characteristics and prenatal diagnosis of a cohort with patients presented GDD/ID with monogenic causes, from the perspective of a tertiary genetic counseling and prenatal diagnostic center.

## Method:

We retrospectively analyzed the diagnostic courses, clinical characteristics, and genetic spectrum of patients presented GDD/ID with rare monogenic causes. We also conducted a follow-up study on prenatal diagnosis in these families. Pathogenicity of variants was interpreted by molecular geneticists and clinicians according to the guidelines of the American College of Medical Genetics and Genomics (ACMG).

## Results:

Among 81 [1] patients with GDD/ID caused by rare monogenic variants it often took 0.5-4.5 years and 2-8 referrals to obtain genetic diagnoses. Developmental delay typically occurred before 3 years of age, and patients usually presented severe to profound GDD/ID. The most common co-existing conditions were epilepsy (58%), microcephaly (21%) and facial anomalies (17%). In total, 111 pathogenic variants were found in 62 different genes among the 81 pedigrees, and 56 variants were novel. The most common inheritance patterns in this outbred Chinese population were autosomal dominant (AD; 47%), following autosomal recessive (AR; 37%), and X-linked (XL; 16%). *SCN2A*, *SHANK3* and *STXBP1* were important causal genes. Hot-spot variants were rarely found. By the follow-up, 33 affected families, including 15, 13 and 5 families inherited in AR, AD and XL modes respectively, had undergone prenatal diagnosis. And the recurrence rates are 26.7%, 15.4% and 20% for families inherited in AR, AD, and XL patterns.

## Conclusion:

Patients presented with GDD/ID caused by rare single gene variants are characterized by early onset, relatively severe symptoms and great clinical variability and genetic heterogeneity. Timely referrals to genetic counseling and prenatal diagnostic laboratories are important for affected families planning to have additional children.

## Introduction

Global developmental delay/intellectual disability (GDD/ID), used to be named as mental retardation (MR) is one of the most common phenotypes in neurogenetic diseases. Global developmental delay (GDD), is characterized by a delay in achieving developmental milestones in at least two of the following

domains: motor skills, speech and language, cognitive skills, and social and emotional skills [1]. After growing up, many patients with GDD would demonstrate intellectual disability (ID), which is characterized by an intelligence quotient below 70 and limitations on adaptability [1, 2]. The prevalence of GDD/ID in the world population is estimated to be 1-3% [3], and the average lifetime costs (direct and indirect) to support an individual with ID have reached \$1 million [4, 5].

Various environmental and genetic factors can result in GDD/ID [6]. Genetic reasons, including aneuploidy, copy number variants and single gene variants, account for 30-50% of cases [7], and Down syndrome, MECP2-related Rett syndrome and fragile X syndrome are the most common forms of genetic GDD/ID [6]. The modes of inheritance of genes related to GDD/ID include autosomal recessive (AR), autosomal dominant (AD), X-linked (XL) and mitochondrial [8]. On the basis of inherited patterns, some scholars divided those genes into ARID (autosomal recessive intellectual disability), ADID (autosomal dominant intellectual disability) and XLID (X-linked intellectual disability) [9-11]. With the improvement of next-generation sequencing (NGS) [12], monogenic genetic causes are being found more frequently in previously unexplained or idiopathic cases. To date, nearly 1334 causative genes and 1159 candidate genes have been identified as related to GDD/ID [13], and the number continues to grow.

With increasing number of patients have obtained the genetic diagnoses by NGS, it poses great challenges on subsequential genetic counseling for family reproduction plan because of our limited knowledge on most rare monogenic diseases. As effective and specific treatments for most monogenic diseases are still in development, and prenatal molecular diagnosis is an important method to prevent recurrence. Previous studies mainly focused on comparing the diagnostic yields of different NGS methods [14] on unidentified GDD/ID or expanding the phenotype and genotype spectrum of a genetic disorder or specific gene. However, the general characteristics of patients presented GDD/ID with rare monogenic causes have not been well studied. Besides, few studies concerned the genetic counseling and prenatal diagnosis.

In this article, we collected a cohort of patients with GDD/ID caused by rare single gene variants. It is the first time, to the best of our knowledge, to observed these patients from the perspective of a genetic counseling and prenatal diagnostic center. The first aim of the study was to describe the diagnostic courses and clinical and genetic spectrum of these patients. The second aim was to report prenatal molecular diagnostic results of affected families, intending to raise awareness on this area.

## **Material And Methods**

### **1. Study design and participants**

From June 2015 to June 2019, we recruited 81 consecutive subjects under 18 years old who presented GDD/ID with rare monogenic causes. The clinical diagnosis of GDD/ID was made according to the Diagnostic and Statistical Manual of Mental Disorders, 5<sup>th</sup> edition (DSM-V) [2]. GDD was defined by

delays in the achievement of motor or mental milestones in the following domains: gross and fine motor skills, speech and language, adaptability and social skills. A developmental scale for children aged 0-5 years[15] was used to assess the Developmental Quotient (DQ) for children who were under 5 years old or failed to finish the intelligence test. Patients with a DQ of less than 70 in at least two of five developmental domains were diagnosed with GDD. For patients over 5 years old, we used the Wechsler Intelligence Scale for Children (WISC) to quantify IQ. Those who had IQ scores lower than 70 and adaptability difficulties were diagnosed with ID. The tests were performed by specialists in child development.

For etiological diagnosis, according to the guideline proposed by American Psychiatric Association (APA) in 2014[1] and Chinese Medical Association(CMA) in 2018[16], all patients underwent systematically examinations, comprising medical history, physical examination, metabolic tests and neuroimaging study (brain MRI/CT) to exclude non genetic causes and underwent necessary genetic tests, such as G-band karyotyping, FMR1 CGG repeat testing [17], and CMA testing [18], to exclude other genetic reasons. Sanger sequencing or Trio-NGS[19, 20], including targeted exome sequencing (panel) or whole exome sequencing (WES) was performed depending on clinical judgment. The details of the detection methods are reported elsewhere.

The final clinical and genetic diagnoses were determined by a group of pediatric neurologists, clinical geneticists and molecular geneticists. The Ethics Committee of Peking University First Hospital approved the study (2020-333). Informed consent was obtained from all participants.

## **2. Data collection**

Demographic data, medical history, laboratory and genetic findings were collected. The severity of GDD/ID was classified into four groups: mild, moderate, severe and profound, defined by DQ (IQ) scores of 55-75 (50-69), 40-54 (35-49), 25-39 (20-34), and below 25 (20), respectively. The age at disease onset was calculated as the interval from the date of birth to the date when the first symptom was noticed. The age at diagnosis was calculated as the interval from the date of birth to the date when genetic diagnosis was confirmed. The interval between symptom onset and diagnosis was obtained as the age at diagnosis minus the age at disease onset. The date of genetic counseling was the time when the patient was referred to outpatient genetic counseling. The duration from genetic counseling to diagnosis was obtained by subtracting the date of diagnosis from the date of genetic counseling.

The normal standardized reference ranges of height, weight and head circumference for children at different ages were obtained from two national growth surveys of children in China[21, 22]. Microcephaly, macrocephaly, short stature and facial anomalies were defined in accordance with the Human Phenotype Ontology (HPO). Positive family history was defined as having family members who presented similar traits to the probands, with or without genetic confirmation. Abnormal birth history was defined as irregular events occurring during delivery or the neonatal period, such as amniotic fluid pollution or neonatal pathological jaundice. Abnormal prenatal ultrasound findings, such as delayed brain

development, biparietal diameter anomaly and intrauterine growth retardation, were also recorded. The last follow-up was in November 2019.

### 3. Criteria for variant interpretation

Standard gene variant nomenclature informed by the Human Genome Variation Society (HGVS)[23] was adopted to unify the description of variants. According to the 2015 American College of Medical Genetics and Genomics (ACMG) guidelines[24], variants were classified as "pathogenic", "likely pathogenic", "uncertain significance (VUS)", "likely benign", or "benign". In order to avoid biases, patients with pathogenic or likely pathogenic variants in known genes were recruited, while patients with VUS variants in known genes or variants in candidate genes were excluded.

For genotype and phenotype comparison, we referred to the Online Mendelian Inheritance in Man (OMIM) database (<https://omim.org/>) and GeneReviews (<https://www.ncbi.nlm.nih.gov/books/NBK11116/>). Allele frequency was searched in two population databases: the Genome Aggregation Database (GnomAD, <https://gnomad.broadinstitute.org/>) and the 1000 Genomes Project (1000G)[25]. The functions of missense variants were predicted in silico with the software programs SIFT[26], Polyphen-2[27], PROVEAN[28] and MutationTaster[29]. The pathogenicity of splicing variants was predicted by the Human Splicing Finder (HSF)[30]. Co-segregation of variants was confirmed in probands and healthy parents, as well as more family members if available, via Sanger sequencing. We searched the Human Genomic Mutation Database (HGMD)[31], ClinVar[32], Ensembl (VEP)[33] and PubMed to determine whether the variant had been reported previously.

### 4. Prenatal diagnostic testing

DNA from chorionic villi or amniotic fluid was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). PCR sequencing was performed using an ABI3730 xl (Applied Biosystems, USA) to detect the causative variants harbored by probands in the family. Linkage analyses with two to five short tandem repeat (STR) markers were performed to exclude contamination with maternal DNA and confirm the originality of a variant.

### 5. Statistical analysis

In this study, all continuous variables were found to be nonnormally distributed; accordingly, they were described as the median (lower quartile, upper quartile) values and compared using the Mann-Whitney U test between two groups. Categorical variables were expressed as frequency rates (percentages). The chi-squared test was used to compare categorical data from at least two groups, and Fisher's exact test was used when the samples were limited. All data analyses were performed using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). A two-sided  $\alpha$  of less than 0.05 was used to define statistical significance.

## Results

As a tertiary genetic counseling and prenatal diagnosis center, our center served 290 families with individuals suspected of rare monogenic diseases during the 4-year study, and 142 (nearly 50%) of those patients had GDD/ID. After excluding 13 patients for missing information, 18 patients for uncertain diagnosis and 3 patients who had pathogenic variants along with atypical manifestations that could not be explained by the variants, we included a total of 108 subjects at preliminary screening. Further reviewing the case history, we excluded 16 patients who reached developmental milestones normally in early stage and suffered GDD secondary to developmental regression, 5 patients that experienced abundant epileptiform activity in the neonatal period and then showed developmental arrest or regression and 7 patients for early death. Finally, we considered a total of 81 subjects and their core family members. (Figure S1).

### **1. Demographic features and diagnostic courses**

The 81 subjects came from 17 out of the 31 provinces and municipalities in mainland China. The numbers of cases inherited in AR, AD, and XL patterns were 30 (37.0%), 38 (47.0%) and 13 (16.0%), respectively. The median age was 50 months (IQR, 25-76.5), and 51 (63.0%) participants were male. The median age of onset was 3.5 months (IQR, 3-7), ranging from the day of birth to 2 years and 3 months. And 70 (95%) participants had symptoms before 1 year, all participants presented GDD before 3 years of age.

The median interval from disease onset to genetic diagnosis was 21 months (IQR, 9-55.5m), ranging from 1 month to 12 years, and the median duration from genetic diagnosis to genetic counseling was 10 months (IQR, 4-23m; range, 0-6.3y). The median number of hospital referrals was 4 (IQR, 2-5; range, 2-8) (Table 1 and Supplementary table 1).

### **2. Clinical characteristics**

Among the 81 subjects, 32 patients were diagnosed as neurodevelopmental disorders (30 syndromic ID and 2 non-syndromic ID), 20 patients had metabolic disorders, 17 patients were genetic epilepsy, and 7 patients had other neurogenetic disorders (3 neuromuscular disorders, 1 developmental brain disorder, 1 genodermatosis, and 1 multiple congenital anomaly). In addition, 54 patients had static courses (GDD had slowly improvement), 13 patients presented progressive courses (GDD followed by psychomotor regression/arrest), and 14 patients of unknown courses. The results were listed in Figure 1, Table 2 and supplementary 2 in detail.

Since developmental scale should be evaluated after 3 months old, GDD might not be their first manifestations. Nearly 25% (22/81) of patients had abnormalities on appearance such as microcephalus, macrocephalus, facial anomalies, short stature, abnormal skin, hair and iris, and kyphoscoliosis, before GDD. Besides 6 patients with development and epileptic encephalopathy presented epilepsy before GDD.

Of the 81 subjects, 15 (22.4%) patients had mild-moderate GDD/ID, and the other 52 (77.6%) had severe to profound GDD/ID. The percentages of patients diagnosed by WES were 47% (7/15) in mild-moderate subjects and 60% (30/50) in severe-profound subjects, the difference of diagnostic methods used in mild and severe patients did not reach statistical significance (Table 5).

Epilepsies were co-existing in 58%(47/81) of patients. Among them, 7 patients had focal epilepsies, 16 had generalized epilepsies, 14 had combined generalized and focal epilepsies and 10 patients were classified as unknown type. Autism spectrum disorders (ASD) were confirmed in 5 patients and 3 patients had borderline ASD.

Other common presentations including facial anomalies (14 [17.2%]), microcephaly (17 [20.9%]), macrocephaly (7 [8.5%]), vision impairment (10 [12.8%]) and hearing loss (4 [5.0%]). Twelve of 60 (24%) patients had low weight, and 5/48 (10%) had short stature. Organ involvements were also observed in 16%(13/81) of subjects: 5 (6%) patients had heart involvements, 3 (4%) had liver involvements, 1 (1%) had kidney involvement, 3 (3.9%) had abnormal skin or hair manifestations, 2 had bone anomalies and 1 patient with thyroid hormone abnormality (Table 2 and 7).

In addition, 48 patients had abnormal brain imaging, the primary abnormalities including cerebral white matter changes (28 [53%]), hypoplasia of corpus callosum (12 [25%]) and cerebellar abnormalities (7 [14.6%]) (Table 6).

The majority of affected individuals (94%, 75/80) were simplex cases (a single occurrence in a family), and only 5 (6%) patients had a positive family history.

Notably, 12.5% (10/80) of patients had abnormal prenatal ultrasound findings. (The details are listed in table 8). In addition, 4 patients experienced hypoxic events during labor, the impact of hypoxic events on their development had been excluded by pediatric neurologists. .

### **3. Genetic characteristics**

In total, 111 pathogenic/likely pathogenic variants were found in 62 different genes among the 81 pedigrees. Of these genes, 28 genes were transmitted in the AR pattern, 25 in the AD pattern and 9 in the XL pattern. In order to analyze the disparity in genetic spectrum between different inherited models, repeated variants were included in the calculation. The results are presented in Table 3 and Supplement 3 in detail. Among these disease-causing variants, there were 51 (45.9%) missense variants, 23 (20.7%) nonsense variants, 24 (21.6%) frameshift variants, 4 (3.6%) small deletion variants, 9 (8.1%) variants that caused splicing defects.

Gene ontology accumulation analyses indicated that those genes took part in multiple biological processes, including nervous system development, nervous impulse transmission, ion transport and metabolism. Genes associated with ion channel transport and nervous system development were mainly inherited in the AD model, while genes related to metabolism were mainly transmitted in AR or XL patterns (Supplementary Figure 2).

Among the 62 different causative genes, *SCN2A*, *SHANK3* and *STXBP1* were found in 3 patients each; and *ALG1*, *CHD2*, *FOXP1*, *GATAD2B*, *GFAP*, *GLB1*, *GRIN2B*, *IDS*, *KCNQ2*, *PAFAH1B1*, *PCDH19*, *SLC9A6* and *SYNGAP1* in 2 patients each. The other 46 out of 62 genes were observed to have pathogenic variants only once each in this cohort.

Most variants were unique in this cohort, while two variants were relatively common. One was the c.1343 A>T in the *GLB1* gene, which occurred in 3 alleles of 2 patients (patient 42/44) among 2 patients with *GLB1*-related diseases. It was a high frequency variant in *GLB1* [34]. The other was a de novo variant c.235C>T in *GFAP*, which was detected in two unrelated patients (Nos.36 and 37) with Alexander Disease. It was a variant that had been reported several times[35-37] but absent in the Normal Population Database (GnomAD and 1000G). Additionally, a homozygous substitution variant, c.1510C>T, was found in patients (Nos. 51) with Tay-Sachs disease, confirmed by hexosaminidase A enzyme deficiency (<1.1nmol/mg/h). Multiple studies[38-40] have reported the pathogenicity of these variants, suggesting that the 1510th base pair in the coding sequence of *HEXA* (NM\_000520) was a common variant position.

Notably, 56 (50.4%) variants were identified as novel variants, and 54 (48.6%) variants have been included in disease databases (ClinVar or HGMD) or reported in PubMed articles. The rate was similar to that in previous studies[41-45]. The proportions of novel variants in ARID, ADID and XLID were 43.8%, 65% and 50%, respectively. This suggests that variant spectrum in known ID genes have not been fully explored in all inheritance patterns. The higher rate of novel variants in ADID might be explained by the fact that most variants arose de novo in the AD pattern.

The major difference among ARID, ADID and XLID lies in the origin of variants. Of the 30 patients with ARID, 27 (90%) patients carried compound heterozygous variants, and 3 (10%) patients harbored homozygous variants. We confirmed that in all patients, the two abnormal alleles were separately inherited from healthy outbred parents who carried the heterozygous variants. Among 38 patients with ADID, 37 (97.4%) variants arose de novo, 1 variant was transmitted from a mosaic father. Of the 13 patients with XLID, 7 (53.8%) patients (2 males, 5 female) had de novo variants, 5 male patients harbored hemizygous variants inherited from their asymptomatic heterozygous mother, and 1 female (patient 70) inherited the heterozygous variant c.445C>T in *PCDH19* from her non-symptomatic father. This unique characteristic was supported by previous reports [46].

In addition, parental somatic mosaicism was found in 2 cases. One is patient 33, who presented with facial dysmorphism and GDD, had a c.941del in *GATAD2B*. The variant was also detected at a low frequency in his paternal peripheral blood genomic DNA but absent in samples of his healthy mother and sister. Therefore, it is likely that the father carries somatic and germline mosaicism for this variant. The other is patient 93, who harbored a hemizygous c.1153C>T in *SLC9A6*. And his mother was suspected to have the variant in mosaic state with a low peak in her peripheral blood Sanger sequencing.

#### 4. Prenatal diagnostic results

By the time of follow-up, totally 33 families underwent prenatal tests to determine whether the next child would harbor the same pathogenic variants as the index patient in the fetal period. As demonstrated in Table 4 and Supplement 4, among them, 15 cases were ARID, 13 cases were ADID and 4 were XLID. And 28 (84.8%) patients chose amniocentesis, and 5 (15.2%) patients underwent chorionic villus sampling. Among the 15 AR cases, 4 fetuses were found to carry two pathogenic variants that originated from parents who were healthy carriers, 9 fetuses harbored one variant, and 2 fetuses did not have any variants. Among the 13 AD cases, 11 fetuses did not have the variants, while 2 fetuses carried the same variants as the proband in the *GATAD2B* gene. Of the 5 XL cases, only 1 fetus harbored the pathogenic variant. The recurrence rates of AR, AD and XL modes were 26.7%, 15.4% and 20% respectively. All variants carried by fetuses were verified after birth or induction of labor.

## Discussion

In this article, we analyzed the diagnostic courses, clinical and genetic characteristics, and prenatal diagnosis of 81 individuals with GDD/ID of monogenic origin. It often took 0.5-4.5 years and 2-8 referrals to obtain a genetic diagnosis after disease onset, reflecting the difficulty of diagnosis. Many factors are associated with this difficulty, including genetic heterogeneity, phenotype and penetrance variability, and shared signs and symptoms. Despite the great variability, when treated as a group of diseases, some features are noteworthy. The empirical findings regarding onset age, severity and coexisting symptoms can be summarized as follows.

One of the distinguishing features is the early age of onset. In our study, all individuals presented developmental delay before 3 years of age, and 80% of them showed abnormal symptoms in the first year of life. Nearly 10% of patients had abnormal during the prenatal stage. This finding is in accordance with previous studies showing that in monogenic forms of ID, the time of onset ranges from the 12th week after conception to early childhood[8] [47]. It also implies that future efforts should be made using NGS in the prenatal stage to detect abnormal prenatal ultrasound findings available and affordable [48, 49].

The severity of GDD/ID ranged from mild to profound in our study, and about 80% of patients had severe to profound disability. This finding is consistent with previous reports that GDD/ID caused by genetic factors could be more severe than those resulting from environmental factors, as the latter are usually mild [50, 51]. Previous studies concluded that de novo variants in ADID genes are the major causes of severe ID, and ARID and XLID are rare in outbred European or Korean populations [41-44], and ARID with homozygous variants is most prevalent in consanguineous populations [45]. In this outbred Chinese cohort, we found that ARID, ADID and XLID had similar rates of severe cases (81%, 80%, and 60%). In addition, ARID with compound heterozygous variants and ADID accounted for approximately 32% and 35.8% of severe cases. Therefore, our results suggested that ARID with compound heterozygous variants also plays an important role in patients with GDD/ID caused by monogenic origin in outbred population. The inconsistency could be partially explained by the difference in study design and population. Another possible explanation is that each person carries 100 to 200 heterozygous private variants that are

potentially deleterious [52], and when asymptomatic and unrelated parents carry such a variant in the same ARID gene, their offspring have a 25% chance of illness.

Most patients (over 95%) had other symptoms besides GDD/ID. Approximately 58% of subjects encompassed epilepsy and 10% had ASD. This finding supports the theory that GDD/IDs share a common etiology with other cognitive and neurological disorders including ASD and seizures [50]. In addition, 43.2% (35/81) of patients manifested abnormalities in appearance, such as microcephalus (21%), macrocephalus (8.6%), facial anomalies (17.2%), short stature (10.4%), and changes in skin or hair (3.8%). The percentage of patients in our cohort who had involvement in other organs, including the heart, kidney, liver, bone and endocrine, was under 16%, which might be lower than the rate in GDD/ID caused by aneuploid.

Genetic heterogeneity was prominent among these patients. Patients with different variants in the same gene could have different manifestations, while patients could have similar phenotype even with different causal genes. For example, the clinical presentation of three patients (No. 88, 89, 90) who were diagnosed with Phelan-McDermid syndrome and carried different frameshift variants in SHANK3 were not exactly similar. Patient 88 and 89 presented profound speech delay and ASD, while facial dysmorphism was found only in patient 88 and epilepsy was found only in patient 89. Patient 90 showed moderate developmental delay and facial dysmorphism but did not have epilepsy or ASD. This finding is consistent with previous studies intended to discover the phenotype-genotype correlation of Phelan-McDermid syndrome[53, 54].

Although experts recommended that when a genetic disorder was highly suspected, the first tier genetic testing is to detect the specific gene directly. We observed that most of our patients were diagnosed with the help of NGS rather than specific gene detection. It suggested the strong power of NGS in diagnosing patients with GDD/ID. We also observed that the percentages of patients diagnosed by WES or panel were similar in this cohort, while WGS is currently not widely used in domestic clinical situations. The best NGS technique for screening patients with GDD/ID remained controversial. It was reported that the diagnostic yields of panel, WES, and WGS in unexplained GDD/ID were up to 11%-32%, 40%, and 42% respectively[43, 44, 55-57]. Previously opinions considered that targeted NGS has the priorities of deeper coverage depth and lower cost than WES, however, hardly any targeted ID-panel could cover a great number of ID genes and chase up to the speed of the discovering new candidate genes, as it is estimated that the number of ID genes is over 1000 and still uprising. Therefore, the choice of personalized diagnostic method relies on the clinicians' experience and varies from person to person.

The second aim of our study was to analyze the prenatal diagnostic situation of these groups of patients. Among the 33 families experienced prenatal diagnosis, the recurrence rates are 26.7%, 15.4% and 20% for ARID, ADID, and XLID, respectively. For GDD/ID caused by variants in autosomal genes, the recurrence rate is determined by whether the origin of the variants is inherited from parents or occurring de novo. For XLID, the recurrence rate is related not only to the originality of the variants but also to the sex of the

fetus, which should be taken into consideration in specific situations. The necessity for prenatal diagnosis of variants inherited from parents has reached consensus in ARID and recessive XLID.

However, with an estimated recurrence rate less than 1%, the question of whether it is necessary to perform prenatal diagnosis for de novo variants in ADID and XLID remains controversial. In this cohort, 2 families with ADID tested positive in prenatal diagnosis, suggesting the high possibility of parental mosaicism in these cases. However, the mosaicism was confirmed in only one (50%) AD family via Sanger sequencing with peripheral blood. Therefore, the recurrence rate of de novo variant in AD families reached 8.3%(1/12), much higher than 1%.

Multiple lines of evidence suggested that the occurrence of parental germline mosaicism is underestimated. Firstly, while previous studies suggested that both Sanger sequencing and exome sequencing have the ability to detect somatic mosaicism [44, 51]. Due to the limitation of sequencing depth and difficulty in specimen acquisition, the existence of mosaicism in asymptomatic parents, which results in an increasing recurrence rate, is usually undetectable at present. Studies using deep amplicon sequencing and digital PCR methods to detect multiple samples found that the proportion of parental mosaicism in some AD or XLID genes reached 5-20% [58-60]. Secondly, nearly half of the ADID and XLID genes in this study have related case reports on germline mosaicism (Supplement 5). Therefore, whether confirmation of parental mosaicism or not, we recommend that prenatal diagnosis is also necessary for de novo variants in ADID and XLID situations. In conclusion, genetic counseling and prenatal diagnostic services are important for families with any ARID, ADID and XLID probands.

Another important observation is that at present, genetic counseling and prenatal diagnostic services are not timely for nearly 50% of families, who were first referred for genetic counseling only after conceiving again. This might result from the lack of awareness and limitation of resources in this field. With the improvement and availability of genetic testing technology, an increasing number of individuals will obtain accurate genetic diagnoses. Additionally, with the implementation of a universal two-child policy[61], the need for genetic counseling and prenatal diagnosis is bound to increase. Therefore, more attention should be paid to this area.

The strengths of our study could be summarized as following. Firstly, it is the first article, to report the general characteristic of a group of patients presented GDD/ID with rare monogenic causes. Secondly, our observations on diagnostic time and methods could be set as baseline data and compared with the that obtained 5-10 years later. Thirdly, via calculating the recurrence rate in a group of families affected by AR, AD and XL genes, our research revealed that the recurrence rate of de novo variant might be underestimated in real world practice. Finally, it is the first retrospective cohort study observed from the perspective of genetic counseling and prenatal diagnostic center, and our result reflected the lack of awareness and shortage of resources in this field.

There are several limitations in our study. One is that rare monogenic neurogenetic diseases with GDD/ID consist of a group of different disorders, and while analyzing it in a cohort, we failed to perform genotype-phenotype correlation of a single syndrome or gene. However, to delineate the relationship in detail, a

group of individuals with variants in the same gene or diagnosed with the same syndrome are needed. This kind of study is restricted by sporadic cases. Additionally, our understanding of these rare monogenic diseases is insufficient, and regular follow-up observations of such patients might provide additional clinical information. Furthermore, as it is a single center study, the results would be influenced by selection bias.

## Conclusion

In summary, individuals with GDD/ID caused by rare monogenic variants are characterized by early onset, relatively severe phenotype as well as great clinical variability and genetic heterogeneity. Patients or pedigrees with such features should be considered to undergo appropriate NGS as early as possible. The spectrum of causal genes and pathogenic variants has not yet been fully discovered. Therefore, clinicians, genetic counselors and genetic laboratories should collaborate tightly to address the problems of diagnosis posed by the bewildering clinical and genetic heterogeneity. Moreover, obtaining clinical and genetic diagnosis is not the final step; timely referral to genetic counseling and prenatal diagnostic laboratories are important for families that plan to have additional children.

## Declarations

### **Ethics approval and consent to participate:**

The Ethics Committee of Peking University First Hospital approved the study (2020-333). Informed consent was obtained from all participants.

### **Consent for publication:**

All authors read the final manuscript and approved for publication.

### **Availability of data and material:**

Most related data and material have been uploaded as supplementary. For more detailed information, please contact the corresponding author.

### **Competing interests:**

All authors declared no conflicts of interests.

### **Funding:**

None.

### **Authors' contributions:**

Liling Lin collected data, performed statistical analysis and wrote the first manuscript. Ying Zhang performed the molecular diagnostic experiments in this study. Jingmin Wang included the participants. Yinan Ma conceived and designed the study and critically revised the manuscript. Hong Pan and Yu Qi supervised the study and critically revised the manuscript.

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## Tables

Table1. Demographic Features

Characteristics	N=81
Male: Female(male%)	51:30(63.0%)
Age, median (IQR), range, m	50 (25, 74), (2, 15y)
Age of onset, median (IQR), m	3.5 (3, 7), (0, 2y3m)
≤1m, n (%)	9 (12.0)
1m<age≤1y, n (%)	61 (79.0)
1y<age≤3y, n (%)	7 (9.0)
Age of genetic diagnosis, median (IQR), range, m	24 (14.5, 72), (3, 12y)
Interval from onset to genetic diagnosis, median (IQR), m	21 (9, 55.5), (1, 12y)
≤6m, n (%)	13 (20.3)
6m<t≤1y, n (%)	11 (17.0)
1y<t≤3y, n (%)	18 (28.0)
3y<t≤5y, n (%)	10 (16.0)
>5y, n (%)	12 (19.0)
Duration from diagnosis to genetic counseling, median (IQR), range, m	10 (4, 23), (0, 6y4m)
Number of referrals, median (IQR), range	4 (3, 5), (2, 8)
Method of diagnosis n (%)	
Whole exome sequencing	43 (56)
Targeted exome sequencing	31 (40)
Sanger sequencing	3 (4)

Abbreviation: IQR, interquartile range; m, months; y, years

Table2.Clinical characteristics

Characteristics	N(%)	Charicteristics	N(%)
<b>Genetic disorders</b>		<b>Abnormal Brain MRI</b>	48/70 (68.5)
<b>Neurodevelopmental Disorders</b>	32/81 (39.5)	<b>Visual impairment</b>	10/78 (12.8)
<b>Metabolism disorders</b>	20/81 (24.7)	<b>Hearing loss</b>	4/78 (5.0)
<b>Genetic epilepsy</b>	17/81 (21.0)	<b>Facial anomalies</b>	14/81 (17.2)
<b>Leukodystrophy</b>	5/81 (6.2)	<b>Head circumference anomaly</b>	24/81 (30)
<b>Other neurogenetic diseases</b>	7/81 (8.4)	<b>Microcephaly</b>	17/81 (20.9)
<b>Disease courses</b>		<b>Macrocephaly</b>	7/81 (8.6)
<b>Static</b>	54/81 (66.6)	<b>Weight</b>	13/50 (26)
<b>Progressive</b>	13/81 (16.0)	<b>Overweight</b>	1/50 (2)
<b>Unknown</b>	14/81 (17.2)	<b>Low weight</b>	12/50 (24)
<b>Severity of GDD/ID</b>		<b>Short stature</b>	5/48 (10.4)
<b>Mild-moderate</b>	15/67 (22.4)	<b>Organ involvement</b>	13/81 (16)
<b>Severe-profound</b>	52/67 (77.6)	<b>heart</b>	5/80 (6.0)
<b>Unknown</b>	14	<b>liver</b>	3/80 (4.0)
<b>Epilepsy</b>	47/81 (58.0)	<b>kidney</b>	1/80 (1.0)
<b>Epilepsy type</b>		<b>hair/skin</b>	3/79 (3.8)
<b>Focal</b>	7/47 (14.9)	<b>bone</b>	2/79 (2.5)
<b>Generalized</b>	16/47 (34.0)	<b>endocrine</b>	1/81 (1.2)
<b>Combined</b>	14/47 (29.8)	<b>Positive family history</b>	5/80 (6.0)
<b>Unknown</b>	10/47 (21.3)	<b>Abnormal antenatal ultrasound</b>	10/80 (12.5)
<b>Autism spectrum disorder</b>	8/80 (10.0)	<b>Abnormal birth history</b>	4/80 (5)

GDD, global developmental delay; ID, intellectual disability;

**Table3. Analysis of genetic spectrum.**

	<b>Total</b>
<b>Number</b>	111
<b>Origin</b>	
paternal	32 (28.8)
maternal	35 (31.5)
de novo	44 (39.6)
<b>DNA change</b>	
substitution	77 (69.4)
deletion	22 (19.8)
duplication	10 (9.0)
insertion	2 (1.8)
<b>Amino acid change</b>	
missense	51 (45.9)
nonsense	23 (20.7)
deletion	4 (3.6)
frameshift	24 (21.6)
splicing defect	9 (8.1)
<b>Loss of function</b>	53 (47.7)
<b>Status</b>	
novel	56 (50.4)
existing	54 (48.6)

AR, autosomal recessive; AD, autosomal dominant; XL, X-linked;

Loss of function variants include nonsense, frameshift, start lost, single or multiple exons deletion and canonical  $\pm 1$  or 2 splice sites.\

#### **Table4. Results of prenatal diagnosis**

	<b>Total</b>	<b>AR</b>	<b>AD</b>	<b>XL</b>
<b>Number of patients</b>	33	15	13	5
<b>Pregnancy status at counseling</b>				
not pregnant	19 (57.5)	11 (73.3)	7 (53.8)	1 (20.0)
pregnant	14 (42.5)	4 (26.7)	6 (46.2)	4 (80.0)
<b>Sample</b>				
Amniotic fluid	28 (84.8)	12 (80.0)	12 (92.3)	4 (80.0)
Chorionic villus	5 (15.2)	3 (20.0)	1 (7.7)	1 (20.0)
<b>Number of variants carried by the fetus</b>				
2	4 (12.1)	4 (26.7)	-	-
1	12 (36.4)	9 (60.0)	2 (15.4)	1 (20.0)
0	17 (51.5)	2 (13.3)	11 (84.6)	4 (80.0)

AR, autosomal recessive; AD, autosomal dominant; XL, X-linked;

**Table 5. Comparison of diagnostic methods between mild and severity patients.**

	<b>Mild-moderate (N=15)</b>	<b>Severe-profound (N=50)</b>	<b>P-value</b>
<b>WES</b>	7(46.7%)	30(60.0%)	0.122
<b>Panel</b>	6(40.0%)	19(38%)	
<b>Sanger</b>	2(13.3%)	1(2.0%)	

Abbreviation: WES, whole exome sequencing; panel, targeted exome sequencing;

**Table 6. Abnormalities of brain MRI in 48 patients**

	N(%)	Gene
<b>Cerebral cortex changes</b>	3 (6.3)	
<b>Lissencephaly</b>	2 (5.2)	<i>PAFAH1B1(2)</i>
<b>Polymicrogyria</b>	1 (2.1)	<i>AKT3</i>
<b>Cerebral white matter changes</b>	28 (58.3)	
<b>Delayed myelination</b>	15 (31.3)	<i>ALG1, BCS1L, FOXP1, GLB1, GNB5, HEXA, IQSEC2, KCNB1, KCNQ2, PMM2, PURA, SHANK3, SLC16A2, TCF4, WDR45</i>
<b>Hypomyelination</b>	10 (20.1)	<i>ATRX, CPLANE1, DDC, GFM1, GJC2, FOLR1C, SMC1A, SOX10, STXBP1, WDR45</i>
<b>Demyelination</b>	3 (6.3)	<i>GALC, GFAP(2)</i>
<b>Cerebral atrophy</b>	4 (8.3)	<i>DOCK6, ERCC8, ISPD, SYNGAP1</i>
<b>Megalencephaly</b>	1 (2.1)	<i>AKT3</i>
<b>Subarachnoid space enlargement</b>	4 (8.3)	<i>GFM1, IDS, IQSEC2, KCNQ2, SCN2A</i>
<b>Hypoplasia of corpus callosum</b>	12 (25)	<i>ATRX, DDX3X, DOCK6, FOXP1, GATAD2B, IQSEC2, PAFAH1B1(2), PMM2, POLR1C, TCF4, ZEB2</i>
<b>Abnormality of cerebellar</b>	7 (14.6)	
<b>Cerebellar atrophy</b>	3 (6.3)	<i>ATP1A3, PMM2, POLR1C</i>
<b>Cerebral dysplasia</b>	4 (8.3)	<i>CPLANE1, ISPD, PMM2, POLR1C</i>
<b>Ventriculomegaly/hydrocephalus</b>	6 (1.3)	<i>AKT3, HEXB, IDS, ERCC8, GALC, GCDH</i>
<b>Basal ganglia lesions</b>	2 (5.2)	<i>ALG1, FARS2</i>

**Table 7. Presentations of organ involvements in 13 patients**

<b>Abnormalities</b>	<b>N</b>	<b>Gene</b>
<b>Heart</b>		
Patent foramen ovale	1	<i>ALG1</i>
Sick sinus syndrome	1	<i>GNB5</i>
Atrial septal defect	1	<i>ISPD</i>
Cardiomyopathy	1	<i>LRPPRC</i>
Congenital heart disease	1	<i>ZEB2</i>
<b>Liver</b>		
Abnormal liver function	2	<i>BCS1L, GLB1</i>
Hepatomagly	1	<i>GCDH</i>
<b>Kidney</b>		
Fanconi syndrome	1	<i>BCS1L</i>
<b>Skin/Hair</b>		
Hypopigmentation; sparse hair, twisted and partial breaks	1	<i>ATP7A</i>
Curly hair, brittle hair	1	<i>BCS1L</i>
Hypopigmented skin patch, cafe-au-lait spots, white hair	1	<i>SOX10</i>
<b>Bone</b>		
Spine (kyphoscoliosis); tooth(hypomature dental enamel)	1	<i>IDS</i>
Abnormal shape of skull	1	<i>IDS</i>
<b>Endocrine</b>		
Thyroid hormone abnormality (FT3↑, FT4↓, T4↓)	1	<i>SLC16A2</i>

**Table 8. Abnormalities of antenatal ultrasound in 10 patients**

Abnormalities	N	Gene
Fetal growth retardation	2	<i>DDX3X, PMM2</i>
Small head circumference	1	<i>CHAMP1</i>
Increasing head circumference	1	<i>GFAP</i>
Hypoplasia of the cerebellar vermis	2	<i>CPLANE1, DOCK6</i>
Enlarged lateral ventricles	1	<i>SPATA5</i>
Hypoplasia of cerebellar vermis and dilation of lateral ventricles	1	<i>ISPD</i>
Congenital heart abnormality	1	<i>ZEB2</i>
Oligohydramnios	1	<i>FARS2</i>

## Figures

Figure 1 is provided in the Supplementary Files section.

### Figure 1

Genetic diagnoses and pathogenic variants in 81 patients presented GDD/ID with rare monogenic causes

## Supplementary Files

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