

Clinical and Genetic Characteristics and Prenatal Diagnosis of Rare Monogenic Global Developmental Delay and Intellectual Disability.

Liling Lin (✉ littlelspage@163.com)

Peking University First Hospital <https://orcid.org/0000-0001-6915-0582>

Ying Zhang

Peking University First Hospital

Hong Pan

Peking University First Hospital

Jingmin Wang

Peking University First Hospital

Yu Qi

Peking University First Hospital

Yinan Ma

Peking University First Hospital

Research

Keywords: monogenic global developmental delay and intellectual disability (GDD/ID), prenatal diagnosis, next-generation sequencing

Posted Date: July 1st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-38415/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published on November 11th, 2020. See the published version at <https://doi.org/10.1186/s13023-020-01599-y>.

Abstract

Background: The estimated worldwide prevalence of global developmental delay (GDD) and intellectual disability (ID) is 1-3%. Rare monogenic GDD/ID is poorly characterized because its low prevalence limits research. In this study, we aimed to describe the diagnostic courses and clinical and genetic characteristics of a cohort with rare monogenic GDD/ID.

Method:

We retrospectively analyzed the diagnostic courses, clinical characteristics, and genetic spectra of rare monogenic GDD/ID patients. We also conducted a follow-up study on prenatal diagnosis in these families. Mutation pathogenicity was interpreted by molecular geneticists and clinicians according to the guidelines of the American College of Medical Genetics and Genomics.

Results:

Among 108 patients with rare monogenic GDD/ID, it often took 0.5-4 years and 3-5 referrals to obtain a genetic diagnosis after disease onset. Onset typically occurred before 6 years of age, and patients usually presented moderate to severe GDD/ID. The most common coexisting conditions were epilepsy (68%), facial dysmorphism (14%) and microcephaly (13%). In total, 149 different pathogenic variants were found in 81 different genes among the 108 pedigrees, and 71 variants were novel. The most common inheritance patterns in this outbred Chinese population were autosomal recessive (AR; 46.3%), autosomal dominant (AD; 37%), and X-linked (XL; 16.7%). *GLB1*, *PLA2G6*, *SCN2A*, *SHANK3* and *STXBP1* were important causal genes. Hot-spot mutations were rarely found. By the follow-up, 43 families, including 24 ARID, 13 ADID and 6 XLID families, had undergone prenatal diagnosis. The offspring of 6 ARID, 2 ADID and 2 XLID families had the same pathogenic variants as the probands.

Conclusion:

Rare monogenic GDD/ID is characterized by early onset, relatively severe symptoms, great clinical variability and genetic heterogeneity. Moreover, timely referrals to genetic counseling and prenatal diagnostic laboratories are important for affected families planning to have additional children.

Introduction

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]. Many patients with GDD 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 monogenic GDD/ID include autosome recessive (AR), autosome dominant (AD), X-linked (XL) and maternal inheritance[8]. On this basis, some scholars divide monogenic GDD/ID into ARID, ADID and XLID[9–11]. With the improvement of next-generation sequencing (NGS)[12], monogenic causes are being found in previously unexplained or idiopathic cases of GDD/ID. Some scholars estimated that the diagnostic yield of exome sequencing in neurodevelopmental delay was 36%, higher than the power of chromosomal microarray (CMA) testing (15–20%)[13]. To date, nearly 1334 causative genes and 1159 candidate genes have been identified as related to GDD/ID[14], and the number continues to grow.

Although numerous new candidate genes and novel variants have been identified, most forms of monogenic GDD/ID have low prevalence, which has led to many challenging problems[15]. One such problem is the difficulty of genetic diagnosis; many GDD/ID patients do not obtain accurate diagnoses. The characteristics of rare monogenic GDD/ID have not been well studied, and a limited number of articles have assessed the disparity among ARID, ADID and XLID. Hence, in this retrospective study, our first aim was to describe the diagnostic courses and clinical and genetic features of a cohort of rare monogenic GDD/ID patients and to explore potential disparities among ARID, ADID and XLID.

In addition, effective and specific treatments for most forms of monogenic GDD/ID are still in development, and prenatal molecular diagnosis is an important method to prevent recurrence. Since studies concerning the prenatal diagnosis of these diseases are rare, the second aim of this study is to report the results of prenatal tests for GDD/ID.

Material And Methods

1. Study design and participants

From June 2015 to June 2019, 108 consecutive subjects under 18 years old with rare monogenic GDD/ID were recruited. The clinical diagnosis of GDD/ID was made according to the Diagnostic and Statistical Manual of Mental Disorders, 5th 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 0–6 years[16] was used to assess the Developmental Quotient (DQ) for children who were under 6 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 6 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, all patients were examined systematically to exclude nongenetic 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] (targeted exome sequencing or whole exome sequencing) 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 or IQ scores of 50–69, 35–49, 20–34, and below 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 dysmorphism 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 for clinical outcome information (i.e., improvement, deterioration, and mortality) 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]. Cosegregation 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[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 Kruskal-Wallis H test among three groups or 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. If differences among three groups reached statistical significance, multiple comparisons were performed using the Bonferroni method. All data analyses were performed using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). A two-sided α of less than 0.05 was used to define statistical significance. For multiple testing, the Bonferroni correction was used to reduce the probability of a type I error, and a two-sided α less than 0.017 (0.05/3) was considered statistically significant.

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 considered a total of 108 subjects (Fig. 1).

1. Demographic features and diagnostic courses of 108 patients with rare monogenic GDD/ID

The 108 subjects came from 21 out of the 31 provinces and municipalities in mainland China. The numbers of ARID, ADID and XLID cases were 50 (46.3%), 40 (37.8%) and 18 (16.2%), respectively. The median age was 41 months (IQR, 25-78.5), and 67 (62%) participants were male. The median age of onset was 6 months (IQR, 3–12), ranging from the day of birth to 5 years and 10 months, and 81 (75%) participants had symptoms before 1 year. All participants presented GDD before 6 years of age, but GDD was not necessarily their first manifestations.

The median interval from disease onset to genetic diagnosis was 14.9 months (IQR, 6–48), ranging from 1 month to 12 years, and the median duration from genetic diagnosis to genetic counseling was 10 months (IQR, 4–23; range, 0-105). The median number of hospital referrals was 4 (IQR, 3–5; range, 1–10) (Table 1 and Supplement 1).

Table 1
Demographic Features

	Total (N = 108)	AR (N = 50)	AD (N = 40)	XL (N = 18)	P value
Male: Female	67: 41	37: 13	22: 18	8: 10	0.044
Age, median (IQR), m	41 (25, 78.5)	37 (19, 73)	46 (28, 74)	53 (27, 112)	
Age of onset, median (IQR), m	6(3, 12)	5 (3,12.5)	6 (2, 9.5)	6 (3, 15)	0.757
≤1 m, n (%)	15 (13.9)	7 (14.3)	5 (13.2)	3 (16.7)	0.653
1m ≤ age ≤ 1y, n (%)	66 (61.1)	30 (61.2)	27 (71.0)	9 (52.9)	
1y ≤ age ≤ 3y, n (%)	21 (19.4)	10 (20.4)	6 (15.8)	5 (29.4)	
3y ≤ age ≤ 6y, n (%)	2 (1.9)	2 (4.0)	0 (0)	0 (0)	
6y ≤ age ≤ 18y, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	
Age of genetic diagnosis, median (IQR), m	24 (14, 60)	20 (14, 53)	28 (15, 72)	48 (14, 96)	0.286
Interval from onset to genetic diagnosis, median (IQR), m	14 .9(6, 48)	12 (6, 33)	21 (6, 60)	39 (9, 88)	0.166
≤6 m, n (%)	28 (25.9)	16 (34)	9 (25.7)	3 (23.1)	0.651
6m ≤ t ≤ 1y, n (%)	16 (14.8)	9 (19.1)	5 (14.3)	2 (15.4)	
1y ≤ t ≤ 3y, n (%)	21 (19.4)	11 (23.4)	9 (25.7)	1 (7.7)	
3y ≤ t ≤ 5y, n (%)	14 (13.0)	6 (12.8)	5 (14.3)	3 (23.1)	
≥5y, n (%)	16 (14.8)	5 (10.6)	7 (20)	4 (30.8)	
Method of sequencing,n (%)					0.967
Whole exome sequencing	52 (48.1)	25 (53.2)	18 (50.0)	9 (56.3)	

Abbreviation: AR, autosomal recessive; AD, autosomal dominant; XL, X-linked; IQR, interquartile range; m, months; y, years

	Total	AR	AD	XL	P value
	(N = 108)	(N = 50)	(N = 40)	(N = 18)	
Targeted exome sequencing	45 (41.7)	21 (44.7)	17 (47.2)	7 (43.8)	
Sanger sequencing	2 (1.9)	1 (2.1)	1 (2.8)	0 (0)	
Duration from diagnosis to genetic counseling, median (IQR), m	10 (4, 23)	9 (3, 23)	16 (5.5, 24.5)	11 (4, 19)	0.506
Number of referrals, median (IQR)	4 (3, 5)	4 (2, 5)	4 (3, 5)	3 (2, 5)	0.605
Abbreviation: AR, autosomal recessive; AD, autosomal dominant; XL, X-linked; IQR, interquartile range; m, months; y, years					

2. Clinical Characteristics

Of the 108 subjects, 7 (7.8%) had mild GDD/ID, and the other 83 (92.2%) had moderate to profound GDD/ID. The common coexisting conditions were epilepsy (68 [63%]), autism spectrum disorder (ASD, 6 [5.5%]), facial dysmorphism (15 [14%]), microcephaly (14 [13.1%]), macrocephaly (8 [7.5%]), hearing loss (7 [6.7%]), and vision impairment (14 [13.5%]). Fifteen of 64 (25%) patients had low weight, and 5/61 (8.2%) had short stature. Compared with ARID and XLID, patients with ADID had an increased rate of ASD (6 [15.4%] vs 0 vs 0) and a decreased rate of brain MRI abnormalities (21[53.8%] vs 40[81.6%] vs 11[73.3%]) (Table 2).

Table 2
Clinical characteristics

	Total		AR		AD		XL		p value
	(N = 108)	%	(N = 50)	%	(N = 40)	%	(N = 18)	%	
Severity of GDD/ID									0.109
mild	7/90	7.8	1/45	2.2	4/31	12.9	2/14	14.3	
moderate	10/90	11.1	4/45	8.9	3/31	9.7	3/14	21.4	
severe	67/90	74.4	40/45	86.7	21/31	67.7	7/14	50.0	
profound	6/90	6.7	1/45	2.2	3/31	9.7	2/14	14.3	
Epilepsy	68/108	63	29/50	58	28/40	70	11/18	61.1	0.496
Autism spectrum disorder	6/110	5.5	0/50	0	6/39	15.4	0/18	0	0.003
EEG abnormality	70/108	64.8	29/50	58	29/40	73.8	12/18	66.7	0.353
Abnormal Brain MRI	72/103	69.9	40/49	81.6	21/39	53.8	11/15	73.3	0.018
Facial dysmorphism	15/107	14	4/49	8.2	7/40	17.5	4/18	22.2	0.221
Visual impairment	14/104	13.5	11/48	22.9	2/39	5.1	1/17	5.9	0.038
Hearing loss	7/105	6.7	6/49	12.2	1/39	2.6	0/17	0	0.140
Head circumference anomaly	22/107	20.6	13/50	26.0	4/36	10.0	5/18	27.8	0.160
microcephaly	14/107	13.1	10/50	20.0	1/36	2.5	4/18	22.2	
macrocephaly	8/107	7.5	3/50	6.0	3/36	7.5	1/18	5.6	
Weight									0.225
Overweight	1/64	1.5	0/28	0	0/27	0	1/9	11.1	
Low weight	16/64	25	9/28	32.1	5/27	12.5	2/9	22.2	
Short stature	5/61	8.2	3/25	12	1/26	3.8	1/10	10	0.372
Organ involvement									-
heart	4/107	3.7	4/51	7.8	0/40	0	0/17	0	0.160
liver	8/107	7.5	7/51	13.7	1/40	2.5	0/17	0	0.078
kidney	1/107	0.9	1/51	2.0	0/40	0	0/17	0	1

AR, autosomal recessive; AD, autosomal dominant; XL, X-linked; GDD, global developmental delay; ID, intellectual disability; EEG, electroencephalogram;

	Total		AR		AD		XL		p value
	(N = 108)	%	(N = 50)	%	(N = 40)	%	(N = 18)	%	
hair/skin	5/108	4.6	2/51	3.9	2/40	5.0	1/18	5.6	1
Positive family history	9/105	8.6	5/49	10.2	2/40	5.0	2/17	11.8	0.581
Abnormal antenatal tests	13/106	12.3	8/50	16.0	4/40	10.0	1/17	5.9	0.556
Abnormal birth history	15/106	14.2	5/50	10.0	8/40	20.0	2/17	11.8	0.442

AR, autosomal recessive; AD, autosomal dominant; XL, X-linked; GDD, global developmental delay; ID, intellectual disability; EEG, electroencephalogram;

Organ involvement was also observed: 5 (4.5%) patients had heart involvement, 10 (9.1%) had liver involvement, 1 (0.9%) had kidney involvement and 5 (4.5%) had abnormal skin or hair manifestations.

The majority of affected individuals were simplex cases (a single occurrence in a family), and only 9 (8.6%) patients had a positive family history. Notably, 13 (12.3%) individuals had abnormal antenatal findings; among them, 8 patients had abnormal prenatal ultrasound results. In addition, 15 (14.2%) patients had an abnormal birth history. These parameters did not show significant differences among the three groups (Table 2). (The details are listed in Supplement 2.)

3. Variant spectra in 108 monogenic GDD/ID patients

In total, 149 different pathogenic variants were found in 81 different genes among the 108 pedigrees. Of these genes, 42 genes were transmitted in the AR pattern, 26 in the AD pattern and 13 in the XL pattern. In order to analyze the disparity in genetic spectra 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 82 (51.9%) missense variants, 30 (19%) nonsense variants, 29 (18.4%) frameshift variants, 5 (3.2%) small deletion variants, 1 (0.6%) multiexon deletion variant and 11 (7%) variants that caused splicing defects.

Table 3
Analysis of genetic spectra

	Total	AR	AD	XL	P Value
Number	158	100	40	18	
Origin					< 0.001
paternal	52 (32.9)	50 (50.0)	1 (2.5)	1 (5.6)	
maternal	56 (35.4)	50 (50.0)	0	6 (33.3)	
de novo	50 (31.6)	0	39 (97.5)	11 (61.1)	
DNA change					0.246
substitution	114 (72.2)	75 (75.0)	27 (67.5)	12 (66.7)	
deletion	29 (18.4)	17 (17.0)	7 (17.5)	5 (27.8)	
duplication	12 (7.6)	6 (6.0)	6 (15.0)	0	
insertion	3 (1.9)	2 (2.0)	0	1 (5.6)	
Amino acid change					0.162
missense	82 (51.9)	55 (55.0)	18 (45.0)	9 (50.0)	
nonsense	30 (19)	17 (17.0)	10 (25)	3 (16.7)	
deletion	5 (3.2)	2 (2.0)	1 (2.5)	2 (11.1)	
insertion	0	0	0	0	
frameshift	29 (18.4)	16 (16.0)	11 (27.5)	2 (11.1)	
splicing defect	11 (7)	9 (9.0)	0	2 (11.1)	
start lost	1 (0.6)	1 (1.0)	0	0	
Loss of function	68 (43.0)	40 (40.0)	21 (52.5)	7 (38.9)	0.383
Status					0.057
novel	76 (48.1)	40 (40.0)	25 (62.5)	10 (55.6)	
existing	82 (51.9)	60 (60.0)	15 (37.5)	8 (44.4)	
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.					

Gene ontology accumulation analyses indicated that those genes took part in multiple biological processes, including nervous system development, nervous impulse transmission, positive regulation of

GTPase activity and energy 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 Fig. 1).

Among the 81 different causative genes, *GLB1* was found in 5 patients; *PLA2G6*, *SCN2A*, *SHANK3* and *STXBP1* in 3 patients each; and *ALG1*, *CDKL5*, *CHD2*, *FOXP1*, *GATAD2B*, *GFAP*, *GRIN2B*, *HEXA*, *IDS*, *KCNQ2*, *PAFAH1B1*, *PCDH19*, *PDHA1*, *SLC9A6* and *SYNGAP1* in 2 patients each. The other 61 out of 81 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 5 alleles of 3 patients (patient 42/43/44) among 5 patients with *GLB1*-related diseases. 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[34–36] but absent in the Normal Population Database (GnomAD and 1000G). Additionally, two homozygous substitution variants, c.1510C > A and c.1510C > T, were found in two patients (Nos. 50 and 51) with Tay-Sachs disease. Multiple studies[37–39] 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, 76 (46.9%) variants were identified as novel variants, and 86 (53.1%) variants have been included in disease databases (ClinVar or HGMD) or reported in PubMed articles. The rate was similar to that in previous studies[40–44]. The proportions of novel variants in ARID, ADID and XLID were 40%, 62% and 50%, respectively. This suggests that variant spectra 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 50 patients with ARID, 44 (88%) patients carried compound heterozygous variants, and 6 (12%) 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 40 patients with ADID, 39 (97.5%) variants arose de novo. Of the 18 patients with XLID, 11 (61.1%) patients (2 male, 9 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 [45].

In addition, parental somatic mosaicism was found in 2 cases. 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. In addition, patient 93 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 diagnosis results

In total, 43 families underwent prenatal tests to determine whether the next child would harbor the same pathogenic variants as the proband in the fetal period. As demonstrated in Table 4 and Supplement 4, among them, 24 cases were ARID, 13 cases were ADID and 6 were XLID. Thirty-six (83.7%) patients chose amniocentesis, and 7 (16.3%) patients underwent chorionic villus sampling. Among the 24 AR cases, 6 fetuses were found to carry two pathogenic variants that originated from parents who were healthy carriers, 13 fetuses harbored one variant, and 5 fetuses did not have any variants. Among the 14 AD cases, 12 fetuses did not have the variants, while 2 fetuses carried the same variants as the proband in the *GATAD2B* gene. Of the 6 XL cases, only 1 fetus harbored the pathogenic variant. All variants carried by fetuses were verified after birth or induction of labor.

Table 4
Results of prenatal diagnosis

	Total	AR	AD	XL	P Value
Number of patients	43	24	13	6	
Pregnancy status at counseling					0.629
not pregnant	26 (56.5)	14 (58.3)	7 (53.8)	2 (33.3)	
pregnant	20 (43.5)	10 (41.7)	6 (46.2)	4 (66.7)	
Sample					0.738
Amniotic fluid	36 (83.7)	19 (79.2)	12 (92.3)	5 (83.3)	
Chorionic villus	7 (16.3)	5 (20.8)	1 (7.7)	1 (16.7)	
Number of variants carried by the fetus					0.001
2	6 (14.0)	6 (25.0)	-	-	
1	16 (37.2)	13 (54.2)	2 (15.4)	1 (16.7)	
0	21 (48.8)	5 (20.8)	11 (84.6)	5 (83.3)	
AR, autosomal recessive; AD, autosomal dominant; XL, X-linked;					

The appropriate time for genetic counseling is before the next pregnancy, owing to the additional procedure to confirm original molecular tests. In this study, 20 (46.5%) families had been pregnant before referral to genetic counseling and prenatal diagnosis, which might influence further management. It has been suggested that, for most families in China in which a proband with a rare monogenic GDD/ID, referral to genetic counseling is usually delayed and reflects a shortage of related resources. Therefore, timely genetic counseling after index patients obtain a genetic diagnosis, should be emphasized to families who plan to have additional children.

Discussion

In this article, we analyzed the diagnostic courses and clinical and genetic characteristics of 108 individuals with rare monogenic GDD/ID. It often took 0.5-4 years and 3–5 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 6 years of age, and 80% of them showed abnormal symptoms in the first year of life. Nearly 10% of patients were 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] [46]. It also implies that future efforts should be made using NGS in the prenatal stage to detect abnormal prenatal ultrasound findings available and affordable [47, 48].

The severity of GDD/ID ranged from mild to profound in our study, and 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 [49, 50]. 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 [40–43], and ARID with homozygous variants is most prevalent in consanguineous populations [44]. However, in the outbred Chinese population, ARID, ADID and XLID had similar rates of severe cases. In addition, ARID with compound heterozygous variants accounted for approximately 50% of severe cases. Our results suggested that ARID with compound heterozygous variants plays an important role in monogenic GDD/ID. 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 [51], and when asymptomatic and unrelated parents carry such a variant in the same ARID gene, their offspring have a 25% chance of illness.

Nearly 90% of individuals in this cohort had manifestations other than GDD/ID. A large proportion of patients, approximately 70% (74/108), encompassed epilepsy and ASD. This finding supports the theory that GDD/IDs share a common etiology with other cognitive and neurological disorders including ASD and seizures [49]. In addition, 32.4% (36/108) of patients manifested abnormalities in appearance, such as short stature, microcephalus, macrocephalus, facial dysmorphism and changes in skin or hair. The percentage of patients in our cohort who had involvement in other organs, including the heart, kidney and skin, was under 10% (11/108), which might be lower than the rate in GDD/ID caused by aneuploid and chromosome structural abnormalities.

Genetic heterogeneity was prominent among the 108 cases, with 149 different variants in 81 genes. Patients with different variants in the same gene could have different manifestations. 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[52, 53].

The second aim of our study was to analyze the prenatal diagnostic situation of these groups of patients. We have helped 46 families with prenatal diagnosis, and the recurrence rates are 25%, 15.4% and 16.7% 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 dominant XLID remains controversial. In this cohort, 2 families with ADID tested positive in prenatal diagnosis, suggesting the possibility of parental mosaicism in these cases. However, it was confirmed in only one AD family via Sanger sequencing with peripheral blood. A previous study suggested that both Sanger sequencing and exome sequencing have the ability to detect somatic mosaicism [43, 50]. However, 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. Multiple lines of evidence suggest that the occurrence of parental germline mosaicism is underestimated. 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% [54–56]. In addition, nearly half of the ADID and XLID genes in this study have related case reports on germline mosaicism (Supplement 5). Therefore, whether confirming parental mosaicism, we recommend that prenatal diagnosis is also necessary for ADID and dominant XLID. In conclusion, genetic counseling and prenatal diagnostic services are important for families with any ARID, ADID and XLID probands.

Another observation is that at present, genetic counseling and prenatal diagnostic services are not timely for nearly 50% of families, who are 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[57], the need for genetic counseling and prenatal diagnosis is bound to increase. Therefore, more attention should be paid to this area.

There are several limitations in our study. First, rare monogenic GDD/ID consists 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. Second, our understanding of these rare monogenic diseases is insufficient, and regular follow-up observations of such patients might provide additional clinical information.

Conclusion

In summary, individuals with rare monogenic GDD/ID 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 correspondence.

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.

Acknowledgements:

We thank all children and parents for participation in this study. We express our gratitude to all pediatric neurologists and other health care providers that involved in diagnosing and treating of these patients.

References

1. Moeschler JB, Shevell M, Committee G on, *Comprehensive evaluation of the child with intellectual disability or global developmental delays*. Pediatrics, 2014. **134**(3): p. e903-18.
2. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders, 5th edn*. Washington, DC: American Psychiatric Association, 2013.
3. Maulik PK, et al. Prevalence of intellectual disability: a meta-analysis of population-based studies. Res Dev Disabil. 2011;32(2):419–36.
4. Department of information, evidence and research. *WHO methods and data sources for global burden of disease estimates 2000–2016*. 2018.
5. Centers for Disease Control and Prevention (CDC). *Economic costs associated with mental retardation, cerebral palsy, hearing loss, and vision impairment—United States*, in *MMWR Morb Mortal Wkly Rep*. 2004. p. 57–59.
6. Flore LA, Milunsky JM. Updates in the genetic evaluation of the child with global developmental delay or intellectual disability. Semin Pediatr Neurol. 2012;19(4):173–80.
7. Srour M, Shevell M. Genetics and the investigation of developmental delay/intellectual disability. Arch Dis Child. 2014;99(4):386–9.
8. Chiurazzi P, Pirozzi F. *Advances in understanding - genetic basis of intellectual disability*. F1000Res, 2016. **5**.
9. Neri G, et al. X-linked intellectual disability update 2017. Am J Med Genet A. 2018;176(6):1375–88.
10. Wiczorek D. Autosomal dominant intellectual disability. Med Genet. 2018;30(3):318–22.
11. Jamra R. Genetics of autosomal recessive intellectual disability. Med Genet. 2018;30(3):323–7.
12. Harripaul R, et al., *The Use of Next-Generation Sequencing for Research and Diagnostics for Intellectual Disability*. Cold Spring Harb Perspect Med, 2017. 7(3).
13. Srivastava S, et al. Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders. Genet Med. 2019;21(11):2413–21.
14. Kochinke K, et al. Systematic Phenomics Analysis Deconvolutes Genes Mutated in Intellectual Disability into Biologically Coherent Modules. Am J Hum Genet. 2016;98(1):149–64.

15. Chiara D, Resta, et al. Next-generation sequencing approach for the diagnosis of human diseases: open challenges and new opportunities. *the Journal of the International Federation of Clinical Chemistry Laboratory Medicine*. 2018;29(1):004–14.
16. Mushi Z, et al., *Standardization of the mental developmental screening test (DST) for children aged 0 ~ 6 years in China*. *Zhonghua Er Ke Za Zhi*, 1997.
17. Chen X, et al. Fragile X syndrome screening in Chinese children with unknown intellectual developmental disorder. *BMC Pediatr*. 2015;15:77.
18. Yi Z, et al. Chromosome Xq28 duplication encompassing MECP2: Clinical and molecular analysis of 16 new patients from 10 families in China. *European Journal of Medical Genetics*. 2016;59(6–7):347–53.
19. Yan H, et al. Targeted next generation sequencing in 112 Chinese patients with intellectual disability/developmental delay: novel mutations and candidate gene. *BMC Med Genet*. 2019;20(1):80.
20. Chen J, et al. Cardio-facio-cutaneous syndrome-associated pathogenic MAP2K1 variants activate autophagy. *Gene*. 2020;733:144369.
21. Coordinating Study Group of Nine Cities on the Physical Growth. and Development of Children and C.I.o. Pediatrics, *A national survey on growth of children under 7 years of age in nine cities of China*. *Chin J Pediatr*. 2007;45(8):609–14.
22. Hui L, et al. Height and weight standardized growth charts for Chinese children and adolescents aged 0 to 18 years. *Zhonghua Er Ke Za Zhi*. 2009;47(7):487–92.
23. den Dunnen JT, et al. HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Hum Mutat*. 2016;37(6):564–9.
24. Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405–24.
25. Genomes Project C, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68–74.
26. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature protocols*. 2009;4(7):1073–81.
27. Adzhubei IA, et al. A method and server for predicting damaging missense mutations. *Nature methods*. 2010;7(4):248–9.
28. Choi Y, et al. Predicting the functional effect of amino acid substitutions and indels. *PLoS one*. 2012;7(10):e46688–8.
29. Schwarz JM, et al. MutationTaster2: mutation prediction for the deep-sequencing age. *Nature methods*. 2014;11(4):361–2.
30. Desmet F-O, et al. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic acids research*. 2009;37(9):e67–7.

31. Stenson PD, et al. The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Human genetics*. 2017;136(6):665–77.
32. Landrum MJ, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic acids research*. 2018;46(D1):D1062–7.
33. Cunningham F, et al. Ensembl 2019. *Nucleic acids research*. 2019;47(D1):D745–51.
34. Caroli F, et al. GFAP mutations and polymorphisms in 13 unrelated Italian patients affected by Alexander disease. *Clin Genet*. 2007;72(5):427–33.
35. Brenner M, et al. Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease. *Nat Genet*. 2001;27(1):117–20.
36. Li R, et al. Glial fibrillary acidic protein mutations in infantile, juvenile, and adult forms of Alexander disease. *Ann Neurol*. 2005;57(3):310–26.
37. Raghavan SS, et al. GM2-ganglioside metabolism in hexosaminidase A deficiency states: determination in situ using labeled GM2 added to fibroblast cultures. *Am J Hum Genet*. 1985;37(6):1071–82.
38. Akli S, et al. Seven novel Tay-Sachs mutations detected by chemical mismatch cleavage of PCR-amplified cDNA fragments. *Genomics*. 1991;11(1):124–34.
39. Paw BH, Wood LC, Neufeld EF. A third mutation at the CpG dinucleotide of codon 504 and a silent mutation at codon 506 of the HEX A gene. *Am J Hum Genet*. 1991;48(6):1139–46.
40. Gieldon L, et al. Diagnostic value of partial exome sequencing in developmental disorders. *PLoS One*. 2018;13(8):e0201041.
41. de Ligt J, et al. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med*. 2012;367(20):1921–9.
42. Han JY, et al. Targeted Next-Generation Sequencing of Korean Patients With Developmental Delay and/or Intellectual Disability. *Front Pediatr*. 2018;6:391.
43. Martinez F, et al. High diagnostic yield of syndromic intellectual disability by targeted next-generation sequencing. *J Med Genet*. 2017;54(2):87–92.
44. Kahrizi K, et al. Effect of inbreeding on intellectual disability revisited by trio sequencing. *Clin Genet*. 2019;95(1):151–9.
45. Samanta D. PCDH19-Related Epilepsy Syndrome: A Comprehensive Clinical Review. *Pediatr Neurol*. 2020;105:3–9.
46. Tau GZ, Peterson BS. Normal development of brain circuits. *Neuropsychopharmacology*. 2010;35(1):147–68.
47. Lord J, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet*. 2019;393(10173):747–57.
48. Petrovski S, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. *Lancet*. 2019;393(10173):758–67.

49. van Bokhoven H. Genetic and epigenetic networks in intellectual disabilities. *Annu Rev Genet.* 2011;45:81–104.
50. Rauch A, et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *The Lancet.* 2012;380(9854):1674–82.
51. Mefford HC, Batshaw ML, Hoffman EP. Genomics, intellectual disability, and autism. *N Engl J Med.* 2012;366(8):733–43.
52. De Rubeis S, et al. Delineation of the genetic and clinical spectrum of Phelan-McDermid syndrome caused by SHANK3 point mutations. *Mol Autism.* 2018;9:31.
53. Kolevzon A, et al. Neuropsychiatric decompensation in adolescents and adults with Phelan-McDermid syndrome: a systematic review of the literature. *Mol Autism.* 2019;10:50.
54. Xu X, et al. Amplicon Resequencing Identified Parental Mosaicism for Approximately 10% of "de novo" SCN1A Mutations in Children with Dravet Syndrome. *Hum Mutat.* 2015;36(9):861–72.
55. Liu A, et al. Mosaicism and incomplete penetrance of PCDH19 mutations. *J Med Genet.* 2019;56(2):81–8.
56. Zhang Q, et al. Genomic mosaicism in the pathogenesis and inheritance of a Rett syndrome cohort. *Genet Med.* 2019;21(6):1330–8.
57. Zeng Y, Hesketh T. The effects of China's universal two-child policy. *The Lancet.* 2016;388(10054):1930–8.

Figures

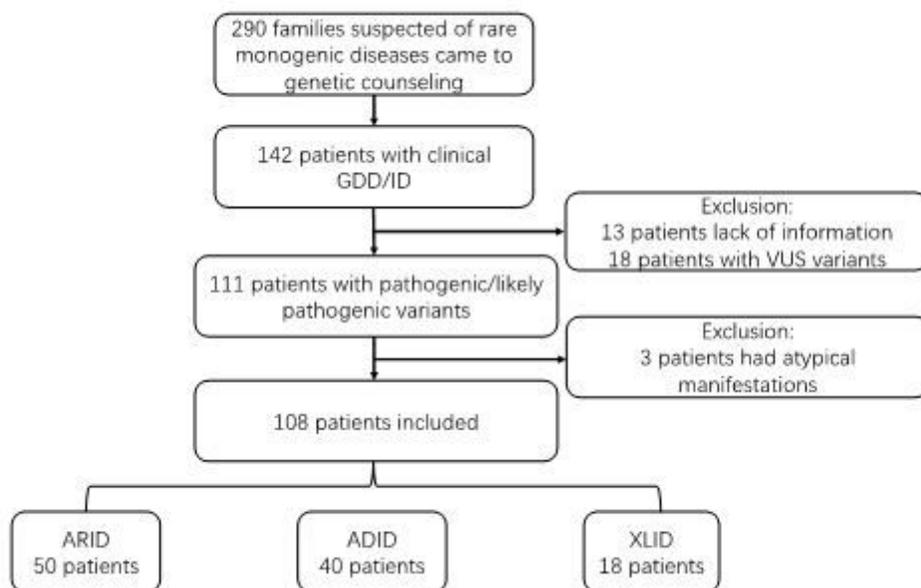


Figure 1

Flowchart of participants' inclusion and exclusion.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementary5.docx](#)
- [Supplementary4prenataltestingresults.xlsx](#)
- [Supplementary3variantspectraandinterpretation.xlsx](#)
- [Supplementary2clinicaldata.xlsx](#)
- [Supplementary1demographicdata.xlsx](#)
- [SupplementaryFigure1.docx](#)