Identification of Multiple Diagnoses in Pediatric Patients through Genome Sequencing

Christin Collins, Fen Guo, Ruby Liu, Yinghong Pan, Mary Colasanto, Madhuri Hegde

1 Introduction

In recent years there has been an increase in the incidence of multiple genetic diagnoses (MGD) reported, largely influenced by advances in next-generation sequencing (NGS) technologies. NGS allows for the simultaneous investigation of an extensive number of genes, the complete exome, and even the entire genome. According to data derived from exome sequencing (ES), the prevalence of MGD varies between 3.5-7.2% in diagnostic testing and 0.4-4% in overall cases. While exome sequencing has offered some insights, genome sequencing (GS), a more comprehensive diagnostic tool, remains underexplored for studying MGD prevalence.

Methods

- Retrospective analysis of pediatric genome sequencing cases referred between March 2018 and September 2022
- No specific inclusion or exclusion criteria
- Consent obtained for testing and return of secondary findings
- Sequence libraries prepared using the NEXTflex Rapid XP kit (Revvity) and sequenced on the NovaSeq 6000 (Illumina, Inc)
- Sequence aligned to human reference GRChr37 (hg19) and variant calling completed using Illumina Bio-IT DRAGEN platform
- Average coverage of nuclear and mitochondrial genomes was 40x and 1000x, respectively
- Repeat expansion analysis for 31 disorders included in analysis, using a “rule out” strategy (included in testing after Nov 2021)
- Screening for spinal muscular atrophy (SMA) included in analysis (included in testing after Nov 2021)

Genetic Diagnoses

- **Definitive Diagnosis (DD):** Any case with a pathogenic or likely pathogenic finding consistent with patient’s clinical presentation
- **Single Definitive Diagnosis (SDD):** Only a single diagnostic finding identified
- **Definitive Multiple Genetic Diagnoses (DMGD):** Two are more pathogenic findings associated with distinct genetic disorders consistent with the patient’s clinical presentation
- **Presumed Multiple Genetic Diagnoses (PMGD):** Pathogenic finding and variant of uncertain significance associated with distinct genetic disorders consistent with the clinical presentation
- **Multiple Genetic Diagnoses (MGD):** Cases categorized as DMGD or PMGD

2 Overall Diagnostic Rate for SDD

	No.	% (95% CI)
Total Cases	245	100.0 (98.5-100.0)
Cases Positive for Diagnostic SNVs (incl. Indels)	198	80.8 (75.4-85.3)
AD Inheritance	106	43.3 (37.2-49.5)
De Novo confirmed	32	13.1 (9.4-17.9)
Inherited from one parent	17	6.9 (4.4-10.8)
No parental studies	57	23.3 (18.4-28.9)
AR Inheritance	62	25.3 (20.3-31.1)
Homozygous, biparental origin confirmed	7	2.9 (1.4-5.8)
Compound Heterozygous, biparental origin confirmed	19	7.8 (5.0-11.8)
Homozygous - no parental studies	23	9.4 (6.3-13.7)
Presumed compound heterozygous - no parental studies	13	5.3 (3.1-8.9)
X-Linked Inheritance	24	9.8 (6.7-14.2)
De Novo confirmed	6	2.4 (1.1-5.2)
Maternally Inherited	7	2.9 (1.4-5.2)
No parental studies	11	4.5 (2.5-7.9)
Mitochondrial Variants	6	2.4 (1.1-5.2)
De Novo confirmed	3	1.2 (0.3-3.5)
Maternally Inherited	1	0.4 (0-2.3)
No parental studies	2	0.8 (0.1-2.9)
Cases Positive for Diagnostic CNVs	40	16.3 (12.2-21.5)
Aneuploidy	5	2 (0.9-4.7)
Copy number gain	10	4.1 (2.2-7.3)
Copy number loss	24	9.8 (6.7-14.2)
Complex rearrangement	1	0.4 (0-2.3)
AR Inheritance, Compound Het for SNV and CNV	3	1.2 (0.3-3.5)
SMA screen positive	2	0.8 (0.1-2.9)
TNR screen positive	2	0.8 (0.1-2.9)

Variant Types Identified in SDD Cases.

Abbreviations: SNV, single nucleotide variant; CNV, copy number variant; AR, autosomal recessive; AD, autosomal dominant; Het, heterozygous; SMA, spinal muscular atrophy; TNR, triplet nucleotide repeat.

A definitive diagnosis was made in 273 of 1,487 patients (diagnostic yield of 18.4%). Of these, 245 were SDD cases (16.5%). The remaining 28 cases were MGD, totaling 1.9% of the cohort.

For SDD cases, 79% of diagnoses were due to SNVs or indels. CNVs accounted for 16.3% of diagnoses for SDD cases. SMA and repeat expansion disorder screening detected 2 positive cases each.

The cohort analyzed includes samples from 1,487 pediatric patients (647 females and 840 males).

	Total	Neonates (<1M)		Infants (1-12M)		Toddlers (13-36M)		Children (3-11Y)		Adolescents (12-18Y)	
	No. (%)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
Total cases	1487 (100)	61	4.1 (3.2-5.2)	241	16.2 (14.2-18.2)	233	15.7 (13.9-17.6)	657	44.2 (41.7-46.7)	295	19.8 (18.0-21.9)
Proband Sex											
Female	647 (43.5)	22	3.4 (2.3-5.1)	121	18.7 (15.9-21.9)	105	16.2 (13.6-19.3)	272	42.0 (38.3-45.9)	127	19.6 (16.8-22.9)
Male	840 (56.5)	39	4.6 (3.4-6.3)	120	14.3 (12.1-16.8)	128	15.2 (13.0-17.8)	385	45.8 (42.5-49.2)	168	20.0 (17.4-22.8)
Case category											
Singleton	711 (47.8)	35	4.9 (3.6-6.8)	135	19.0 (16.3-22.0)	114	16.0 (13.5-18.9)	293	41.2 (37.6-44.9)	134	18.8 (16.1-21.9)
Trio	776 (52.2)	26	3.4 (2.3-4.9)	106	13.7 (11.4-16.3)	119	15.3 (13.0-18.0)	364	46.9 (43.4-50.4)	161	20.7 (18.0-23.7)
Sample Type											
DBS	200 (13.4)	12	6.0 (3.5-10.2)	49	24.5 (19.1-30.9)	42	21.0 (15.9-27.2)	79	39.5 (33.0-46.4)	18	9.0 (5.8-13.8)
Saliva	632 (42.5)	6	0.9 (0.4-2.1)	53	8.4 (6.5-10.8)	97	15.3 (12.7-18.4)	309	48.9 (45.0-52.8)	167	26.4 (23.1-30.0)
Whole blood	461 (31.0)	38	8.2 (6.1-11.1)	112	24.3 (20.6-28.4)	63	13.7 (10.8-17.1)	173	37.5 (33.2-42.0)	75	16.3 (13.2-19.9)
gDNA	194 (13.0)	5	2.6 (1.1-5.9)	27	13.9 (9.7-19.5)	31	16.0 (11.5-21.8)	96	49.5 (42.5-56.5)	35	18.0 (13.2-24.1)
Previous testing											
With prior genetic testing	694 (46.7)	11	1.6 (0.9-2.8)	88	12.7 (10.4-15.4)	122	17.6 (14.9-20.6)	330	47.6 (43.9-51.3)	143	20.6 (17.8-23.8)
Without prior genetic testing	793 (53.3)	50	6.3 (4.8-8.2)	153	19.3 (16.7-22.2)	111	14.0 (11.8-16.6)	327	41.2 (37.9-44.7)	152	19.2 (16.6-22.1)
Secondary Findings											
ACMG Secondary Findings	1145 (77.0)	33	2.9 (2.1-4.0)	171	14.9 (13.0-17.1)	181	15.8 (13.8-18.0)	526	45.9 (43.1-48.8)	234	20.4 (18.2-22.9)
Other Diagnostic Findings	869 (58.4)	24	2.8 (1.9-4.1)	113	13.0 (10.9-15.4)	129	14.8 (12.6-17.4)	425	48.9 (45.6-52.2)	178	20.5 (17.9-23.3)
Carrier Status	884 (59.4)	24	2.7 (1.8-4.0)	110	12.4 (10.4-14.8)	132	14.9 (12.7-17.4)	436	49.3 (46.0-52.6)	182	20.6 (18.1-23.4)
Pharmacogenetic Variants	881 (59.2)	23	2.6 (1.7-3.9)	106	12.0 (10.0-14.3)	141	16.0 (13.7-18.6)	425	48.2 (45.0-51.5)	186	21.1 (18.5-23.9)

Distribution of demographic information across samples tested. M: months, Y: years, CI: confidence interval, DBS: dried blood spots, gDNA: genomic DNA, ACMG: American College of Medical Genetics and Genomics

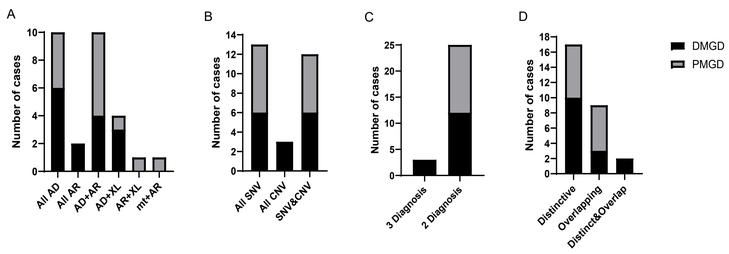
3 Landscape of multiple genetic diagnoses cases

For MGD cases, diagnoses in 13 of the 28 MGD cases were exclusively due to SNVs, while 12 received diagnoses resulting from a combination of SNVs and CNVs.

A single mode of inheritance was noted in 12 of 28 cases, while 16 showed multiple inheritance types.

Most had one additional diagnosis (25/28 MGD cases); however, 3 of 28 MGD cases had three distinct diagnoses.

Of the MGD cases: 17 presented with phenotypic features of each diagnosis, 9 showed features that overlapped between their diagnoses, and 2 displayed a mix of distinct and overlapping clinical features.



The landscape of the MGD cases: A) inheritance patterns, B) variant types, C) diagnosis numbers, and D) phenotypic complexity. Abbreviations: AD, autosomal dominant; AR, autosomal recessive; XL, X-linked; mt, mitochondrial; SNV, single nucleotide variants; CNV, copy number variants; DMGD, Definitive Multiple Genetic Diagnoses; PMGD, Presumed Multiple Genetic Diagnoses

4 Variables affecting diagnostic yield

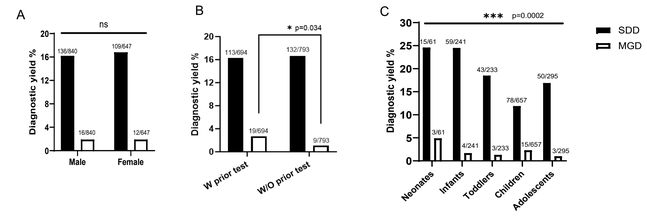
Diagnostic yield showed no difference between male and female patients in SDD and MGD cases. For SDD cases, the yield was 16.2% for males and 16.8% for females. For MGD cases it was 1.9% in both males and females.

For SDD cases, diagnostic yield was unrelated to previous testing: 16.3% with prior testing and 16.6% for those without.

For MGD cases, individuals with prior testing showed a 14.4% diagnostic yield compared to 6.4% for those with GS as a first-tier test.

For 19 MGD cases with prior testing: 17 lacked a complete diagnosis, 8 had partial findings, 9 were non-diagnostic.

Age at testing also impacted diagnostic yield. Neonates and infants exhibited the highest diagnostic yield for SDD at 24.6% and 24.5%, respectively. For MGD cases, neonates show elevated diagnostic yield at 4.9%.



*Variables affecting diagnostic yield and the complex genetic landscape of multiple genetic diagnoses. A) Diagnostic yield vs sex. B) Diagnostic yield vs with (W) or without (WO) previous testing. C) Diagnostic yield vs age group. Abbreviations: SDD, Single Definitive Diagnosis; MGD, Multiple Genetic Diagnoses; ns, not significant; * p-value < 0.05; *** p-value < 0.001.*

5 Case Presentations of MGD

Of the 28 MGD cases, 15 were DMGD and 13 were PGMD.

Here we present a summary of 3 DMGD and 2 PGMD cases identified:

Submitted Clinical Presentation	Prior Genetic Testing	Molecular Mechanism	OMIM Dx / Related Features	Overlapping/ Distinctive	Genetic Diagnosis
3-year-old female with short stature, brachydactyly, macrocephaly, progressive lumbar kyphosis, very short extremities, shortening of tubular bones of both extremities appears with widening and lower lumbar lordosis, febrile seizures, concern for achondroplasia and hypochondroplasia.	External karyotype and panel testing: non-diagnostic	COL2A1 c.2257G>A p.(Gly753Ser) Heterozygous, Pathogenic	COL2A1-related skeletal dysplasia / short stature, brachydactyly, macrocephaly, progressive lumbar kyphosis, very short extremities, shortening of tubular bones of both extremities appears with widening and lower lumbar lordosis	Distinctive	DMGD
		PCDH19 c.2571_2572del p.? Heterozygous, Pathogenic	Developmental and epileptic encephalopathy 9 / febrile seizures		
1-month-old female with non-immune fetal hydrops, dysmorphic facial features (low-set ears and up-slanting palpebral fissure) concerning for Down syndrome, skeletal dysplasia, Noonan syndrome, severe edema/ascites, hypotension, hypothyroidism, elevated amino acid, SCID, large perimembranous VSD, persistent pulmonary hypertension of newborn, single palmar crease on left hand, rhizomelic shortening of upper and lower extremities, polyhydramnios, premature birth.	None	33.7 Mb dup of 21q11.2q22.3 Pathogenic	Down syndrome / dysmorphic facial features (low set ears and up-slanting palpebral fissure), severe edema/ascites, hypotension, hypothyroidism, large perimembranous VSD, single palmar crease on left hand, rhizomelic shortening of upper and lower extremities	Distinctive	DMGD
		GLA c.679C>T p.(Arg227Ter) Heterozygous, Pathogenic	Fabry disease; Fabry disease, cardiac variant / too early to manifest		
6-year-old female with dysgenesis of corpus callosum, muscular hypotonia, microcephaly, delayed motor development, delayed language development, intellectual disability, attention deficit disorder, elevated liver enzymes, coloboma, microphthalmia, strabismus, visual impairment, hypodontia, dysmorphic facial features, hypotelorism, upslanting palpebral fissure, low set protruding posterior rotated ears, midface hypoplasia, prominent everted upper lips, small chin, small nails, clinodactyly, overlapping toes, failure to thrive, IUGR, oligohydramnios. Clinical features manifested in infancy.	External karyotype: non-diagnostic	THOC6 c.810+1G>A p.? Homozygous, Likely Pathogenic	Beaulieu-Boycott-Innes syndrome / microcephaly, delayed motor development, delayed language development, intellectual disability, dysmorphic facial features, upslanting palpebral fissure, prominent everted upper lips	Distinctive; Overlapping feature: visual impairment	DMGD
		ABCA4 c.5882G>A p.(Gly1961Glu) Homozygous, Pathogenic with reduced penetrance	ABCA4 -related ocular disorders; Retinitis pigmentosa 1 / visual impairment		
		RP1 c.5248G>T p.(Glu1750Ter) Homozygous, Likely Pathogenic			
9-month-old male with severe growth and developmental delay, failure to thrive, Romano-Ward syndrome, hx of G-tube feeding, metabolic disorder, mitochondrial metabolic disorder, agenesis of corpus callosum, seizure disorder, hypertension, irregular breathing pattern, hypovia, lactic acidosis, acute respiratory failure with hypoxia and hypercapnia, respiratory distress, respiratory insufficiency, half-sibling with autism.	External WES: KCNH2 variant and SLC25A19 variants reported	KCNH2 c.774_789del p.? Heterozygous, Pathogenic	Long QT syndrome 2; Short QT syndrome 1 / Romano-Ward syndrome	Distinctive	PGMD
		SLC25A19 c.115A>T p.(Ile39Phe) Heterozygous, VUS	Microcephaly, Amish type; Thiamine metabolism dysfunction syndrome 4 / severe growth and developmental delay, lactic acidosis		
		SLC25A19 c.433C>A p.(Arg145Ser) Heterozygous, VUS			
8-year-old-female with mixed conductive and sensorineural hearing loss, short stature, bilateral hypermetropia, bilateral 5th finger brachydactyly, bilateral little finger clinodactyly, hoarseness. Clinical features manifested at 6 months	External panel testing: MYO3A VUS reported	ANKRD11 c.2288_2289del p.? Heterozygous, Pathogenic	KBG syndrome / short stature, bilateral 5th finger brachydactyly, bilateral little finger clinodactyly	Distinctive	PGMD
		TNC c.5692G>A p.(Val1898Ile) Heterozygous, VUS	Deafness 56 / hearing loss		

6 Conclusions

Of 1,487 pediatric patients tested, a definitive diagnosis was returned for 273 patients (18.4%). Of these, 245 were SDD cases comprising 16.5% of the total cohort and 89.7% of the diagnosed cases. The remaining 28 cases were MGD, totaling 1.9% of the cohort and 10.3% of the diagnostic cases. The MGD cases include 15 DMGD cases and 13 PMGD cases.

This study solely utilizes genome sequencing to assess the prevalence of MGD. Our findings highlight the complexity of rare diseases and underscore the critical role of a comprehensive, genome-level diagnostic approach. Even with an initial diagnosis, clinicians should ensure it fully explains the observed phenotype to guide optimal therapeutic strategies and management.

Revvity Inc., 940 Winter Street, Waltham, MA USA (800) 762-4000 or (+1) 203 925-4602 www.revvity.com