

# Genetic Evaluation of Global Developmental Delay in Children: A Series of Seven Cases

PRAMILA GOPAL MENON<sup>1</sup>, RENUKA MAJJIGUDDA<sup>2</sup>, SNEHA SAGARKAR<sup>3</sup>,  
PARAG TAMHANKAR<sup>4</sup>, SHAILAJA VIJAY MANE<sup>5</sup>



## ABSTRACT

Global Developmental Delay (GDD) is a multifaceted and varied condition primarily linked to genetic factors. Due to its heterogeneous nature, multitiered investigations are typically recommended to identify the underlying causes and create an effective intervention plan. This study evaluated the Single Nucleotide Variants (SNVs) associated with GDD in children from Maharashtra, India. Whole Exome Sequencing (WES) was conducted on blood samples from seven children referred to Dr. D. Y. Patil Medical College Hospital and Research Centre, Pune, Maharashtra, India for unexplained GDD/Intellectual Disability (ID) between January 2020 and March 2022. The ages of the seven patients in this study ranged from three months to 14 years. Out of the seven cases, five exhibited variants in the CHRNG, FUCA1, MECP2, GNRHR, AKT3, and PTCH1 genes. In two instances, novel variants of likely pathogenic or uncertain significance were identified in seven genes involved in neurodevelopment and immunity. These results emphasise the importance of genetic screening in diagnosing GDD, thereby enabling targeted interventions. Additional studies with larger cohorts are required to confirm these findings and determine their clinical relevance.

**Keywords:** Developmental disabilities, Exome sequencing, Paediatric

## INTRODUCTION

The GDD/ID refer to significant delays in the acquisition of developmental milestones in a child's growth across key domains such as motor skills, speech and language, cognition and social abilities [1]. Although the worldwide prevalence of developmental delay in paediatric populations under 14 years is estimated to be around 5%, in India, the prevalence varies from 3% to 13% (Juneja M et al., 2022) [2]. Moreover, a study conducted at a Primary Health Centre in Maharashtra found a higher prevalence of 11% among children aged one to three years [3]. Most children with GDD exhibit cognitive impairment that affects their social, emotional and behavioural skills. Genetic defects are the most prevalent cause, accounting for approximately 30-50% of cases. Among these, the burden of gene-disrupting, de novo, and inherited mutations is highest in neurodevelopmental genes [4,5]. Therefore, accurately identifying the underlying cause will be essential for directing precise treatment and improving children's health. This study aimed to investigate the genetic underpinnings of GDD observed in seven children referred to Dr. D.Y. Patil Hospital and Research Centre and correlate them with their clinical observations.

Multiple genetic factors, such as Single Nucleotide Variants (SNVs), insertions/deletions (indels), and copy number variations, are key players in the pathogenesis of GDD but are not fully characterised. The majority of disease-causing variants are located in exons; hence, Whole Exome Sequencing (WES) effectively diagnosis complex genetic disorders like GDD [6,7]. Compared to conventional methods, WES analysis detects multiple known SNVs and indels (nucleotides) in genes such as Gamma-Aminobutyric Acid Type A Receptor Subunit Gamma2 (GABRG2), Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate 2B (GRIN2B), Activity-Dependent Neuroprotective Protein (ADNP), and Methyl CpG Binding Protein 2 (MECP2). It also uncovers novel variants in genes linked to neurodevelopment [4]. WES is recommended as a first-tier clinical diagnostic test for neurodevelopmental disorders [8,9]. Exome sequencing has elucidated monogenic forms of GDD/ID that are not detectable by chromosomal microarray, fragile X syndrome testing, or single-gene sequencing of MECP2 or Phosphatase and Tensin Homolog (PTEN) [10,11].

In this study, WES was performed on seven children with unexplained GDD/ID. Three previously characterised and four novel SNVs/indels were identified, suggesting the potential of WES in identifying the molecular aetiology of GDD.

## CASE SERIES

This series included seven children with unexplained GDD and ID, who were referred to our hospital from January 2020 to March 2022. The Institutional Ethics Committee (IEC) of Dr. D. Y. Patil Vidyapeeth, Pune, approved this series under reference no. DYPV/EC/612/2020. As the patients were minors, written consent was obtained from each patient's parents. The children were diagnosed by a paediatrician, paediatric neurologist, or geneticist. The characteristics of each patient, including age, gender, clinical observations and phenotypes, are listed in [Table/Fig-1].

Genomic DNA was extracted from the blood according to the manufacturer's instructions (QIAamp DNA Blood MiniKit). A Qubit spectrophotometer (Thermo Scientific, USA) was used to assess the quality of the samples. The library for exome sequencing was prepared with 500 µg of DNA using the Illumina DNA Prep with Exome 2.0 Plus Enrichment kit, following the manufacturer's instructions. Sequencing of the protein-coding regions of approximately 30 Mb of the human exome (targeting approximately 99% of areas in CCDS and RefSeq) was performed using Illumina Next-Generation Sequencing (NGS) at a mean depth of 50-60X, with over 90% of the bases covered at a 20X depth in the target region. In some cases, due to the complexity of the sequence, not all variants in the flanking regions were analysed. A base is considered to have sufficient coverage at 20X, and an exon is fully covered if all coding bases plus three nucleotides of flanking sequence on either side are covered at 20X or more.

The GATK best practice framework was followed for variant identification. A BWA-mem aligner was used to align the sequences obtained with the human reference genome (GRCh37/hg19). Duplicate reads and those with base quality scores below Q30 were excluded, and re-alignment of reads was performed based on insertions and deletions (indels) using Sentieon's inbuilt modules [12]. Quality Control (QC) metrics included total SNVs and indels,

Case No.	Age/sex	Birth order/	Type of delivery	Clinical features	Investigations
1	9 y/F	Non consanguineous	Normal delivery	GDD, wandering eyes, seizures and cerebellar signs	Clinically suspected for Rett syndrome and other associated disorders.
2	3 m/F	Non consanguineous	Normal delivery, ANC/polyhydramnios and intrauterine growth restriction	GDD, Platyccephaly, low set ears, rocker bottom feet, hypertrichosis, bluesclera, bowing of legs, limb abnormalities, joint restriction, multiple joint contractures, short stature and dysmorphic facial features	Clinically suspected to have Escobar Syndrome, Congenital heart disease murmur plus, karyotyping normal, Echo Atrial Septal Defect (ASD), Ultrasound (USG) head normal.
3	6 y/M	Consanguineous	Normal delivery	GDD, hyperactivity, behavioural issues, microcephaly, inadequate socio-adaptive functioning, intellectual disability, mild autism, epicanthal folds, long philtrum, supernumerary nipples, hypospadias, kyphosis, pes planus and congenital radioulnar synostosis	Magnetic Resonance Imaging (MRI) showed prominent Virchow-Robin (VR) spaces. Visual Evoked Potential (VEP) suggestive of prolonged P100 latency in both eyes and pigmentary retinal changes. Clinically suspected to have Costello syndrome, Pallister Killian syndrome, and 3- M syndrome.
4	14 y/M	Consanguineous	Normal delivery	GDD, congenital heart disease, renal tubular acidosis, short stature, coarse facies, Dolicocephaly head, normal eyes	Echo Mitral regurgitation, MRI brain periventricular leukomalacia, T2 hypointensities in Globus pallidum, Urine Mucopolysaccharidoses (MPS) - Glycosaminoglycans (GAG) positive. Clinically, suspected for mucopolysaccharadosis.
5	10 y/M	Consanguineous	Normal delivery	GDD, first episode of generalised tonic-clonic seizures at 5 <sup>th</sup> month of life along with fever. At 9 years, early manifestations of metachromatic leukodystrophy	EEG showed abnormal slowing of activity suggestive of diffuse encephalopathy and abnormal epileptic discharge at right temporal region. Urine analysis suggested 3 methyl glutaric aciduria ketosis, dicarboxylic aciduria, 3 hydroxy carboxylic aciduria.
6	7 m/F	Non consanguineous	Normal delivery	GDD, no neck holding, no vocalisation, mongoloid spots and squint, Low tone, reflex +++, bilateral plantar reflex low	The patient was lost to follow-up.
7	4 y/M	Non consanguineous	Oligohydramnios, NICU admission Ventilator therapy	Fever, cough, cold, failure to gain weight, constipation and dental caries. Had dysmorphic features including triangular face, low set ears and retrognathia	2D ECHO suggestive of mild pulmonary arterial hypertension secondary to lower respiratory tract infection. USG suggestive of Cystitis.

[Table/Fig-1]: Characteristics of patients in this study.

annotated variant categories, and heterozygous alternative alleles (genotype probability >0.8). Clinically relevant variants were identified using Sentieon's HaplotypeCaller and DeepVariant pipelines on Google Cloud [13]. Variant annotation was performed using the OMIM, GWAS, GNOMAD, and 1000 Genomes databases [14-17]. SNVs and copy number variants were detected using the clinically validated ExomeDepth (v1.1.10) and classified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) [18].

As shown in [Table/Fig-1], the ages of the seven patients in this study ranged from three months to 14 years. The male-to-female ratio was 4:3. No bias was observed concerning sex, mode of delivery, gestational age and birth weight [Table/Fig-1]. The molecular diagnostics for the seven patients are provided in [Table/Fig-2]. Based on the type of mutation, patients were classified into three groups.

Three patients had a single pathogenic, well-characterised mutation in the MECP2, Cholinergic Receptor Nicotinic Gamma Subunit (CHRNG),

and Gonadotropin-Releasing Hormone Receptor (GNRHR) genes, respectively. Additionally, two patients had novel variants in alpha-L-fucosidase 1 (FUCA1) and patched 1 (PTCH1) that were likely pathogenic. The other two patients had multiple variants related to their clinical presentations, which were predicted to be damaging by various prediction tools [Table/Fig-3]. A description of the individual cases is provided below:

**Case 1:** A nine-year-old was noted to have developmental regression, clinical observations indicating GDD, wandering eyes, seizures and cerebellar signs [Table/Fig-1,4]. Whole Exome Sequencing (WES) revealed that the patient had a c.916 C>T (p.Arg306Cys) mutation in exon 4 of the MeCP2 gene, which is associated with Rett syndrome [Table/Fig-2].

**Case 2:** A three-month-old presented with platycephaly, low-set ears, rocker-bottom feet, hypertrichosis, blue sclera, bowing of the legs, and limb abnormalities indicative of multiple pterygium syndrome.

Case No.	Age/sex	WES- Variant	Variant and chromosomal location	Zygosity	Inheritance	Syndrome	ACMG classification of variant	ACMG criteria for pathogenicity	Reported/ Novel
1	9 y/F	NM_004992.4, MECP2, Exon 4	c.916C>T (p.Arg306Cys) chrX-153296363 G>A	Heterozygous	X-linked Dominant	Rett syndrome (312750)	Pathogenic (II)	PS4, PS3, PS2, PM1, PM2, PM5, PP1, PP3, PP5	Reported
2	3 m/F	NM_005199.5, CHRNG, Exon 7	c.753_754delCT (p.Val253Alafs*44) chr2-233407739 CCT>C	Homozygous	Autosomal Recessive	Multiple pterygium syndrome, lethal type (253290)	Pathogenic (Ib)	PVS1, PM2, PM3	Reported
3	6 y/M	NM_000406.3, GNRHR, Exon 3	c.785G>A (p.Arg262Gln), chr4-68606400 C>T	Heterozygous	Autosomal Recessive	Hypogonadotropic hypogonadism 7 with or without anosmia (146110)	Pathogenic (IV)	PM1, PM2, PM3, PM5, PP2, PP3	Reported
4	14 y/M	NM_000147.5, FUCA1, Exon 1	c.262C>T (p.Gln88Ter), chr1-24194515 G>A	Homozygous	Autosomal Recessive	Fucosidosis (230000)	Likely Pathogenic (I)	PVS1, PM2	Novel
5	10 y/M	NM_005465.7, AKT3, Exon 14 NM_001083603.3, PTCH1, Exon 1	c.1393C>T, (p.Arg465Trp), chr1-243668598 G>A c.169delA, (p.Arg57Glyfs*22) chr9-98278933 CT>C	Heterozygous Heterozygous	Autosomal Dominant Autosomal Dominant	Megalencephaly-polymicrogyria polydactyly-hydrocephalus syndrome 2 (615937) and Basal cell nevus syndrome 1 (109400)	Likely Pathogenic (II) Likely Pathogenic (I)	PS2, PS3, PM2, PP2 PVS1, PM2 (0.05%)	Reported Novel

6	7 m/F	NM_001170535.3, ATAD3A, Exon 6, NM_003496.4, TTRAP, Exon 24, NM_001007026.2 ATN1, Exon 7	c.526C>T, (p.Arg176Trp) chr1-1455532 C>T  c.3257G>A, (p.Gly1086Glu) chr7-98527693 G>A  c. 3057G>C, (p.Glu1019Asp) chr12-7048183 G>C	Heterozygous Heterozygous Heterozygous	Autosomal Dominant Autosomal Dominant Autosomal Dominant	Harel-Yoon syndrome (617183),  Developmental delay with or without dysmorphic facies and autism (618454),  Congenital hypotonia, epilepsy, developmental delay and digital anomalies (618494)	Uncertain Uncertain Uncertain	PM2 PM2, PP2 PM2, BP4	Novel Novel Novel
7	4 y/M	NM_001354930.2, RIPK1, Exon 11, NM_170606.3, KMT2C, Exon 52	c. 1808G>A, (p.Arg603His) chr6-3113365 G>A  c.13174C>T, (p.Pro4392Ser) chr7-151845838 G>A	Heterozygous Heterozygous	Autosomal Recessive Autosomal Dominant	Immunodeficiency 57 with autoinflammation (618108),  Kleefstra syndrome 2 (617768)	Uncertain Uncertain	PM2, PP3 PM2	Novel

[Table/Fig-2]: Details of variants observed in this study.

Variant	Functional coding					Function whole genome		
	Reval	SIFT	Polyphen2	Mutation Taster (MT)	DANN	BayesDel	GenoCanyon	fitCons
FUCA1: c.262c>T	(N/A)	(N/A)	(N/A)	Deleterious (1)	Deleterious (0.98)	Deleterious (strong) 0.66	Deleterious (1)	Deleterious (0.65)
AKT3: c.1393C>T	Uncertain (0.4)	Uncertain (0.003)	(N/A)	Deleterious (1)	Deleterious (1)	Deleterious (supporting) (0.19)	Deleterious (1)	Deleterious (0.72)
PTCH1: c.169delA	(N/A)	(N/A)	(N/A)	(N/A)	(N/A)	(N/A)	(N/A)	(N/A)
ATAD3A: c.526C>T	Benign (moderate) (0.26)	Uncertain (0.017)	Benign (supporting) (0.02)	Deleterious (0.94)	Deleterious (0.99)	Uncertain (-0.12)	Deleterious (0.74)	Deleterious (0.71)
TTRAP: c.3257G>A	Uncertain (0.47)	Uncertain (0.065)	(N/A)	Deleterious (1)	Deleterious (1)	Deleterious (supporting) (0.21)	Deleterious (1)	Deleterious (0.71)
ATN1: c.3057G>C	Benign (moderate) (0.04)	Benign (Supporting) (0.249)	(N/A)	(Deleterious) 0.97	(Deleterious) 0.68	Benign (moderate) (-0.42)	Deleterious (1)	Deleterious (0.72)
RIPK1: c.1808G>A	Deleterious (Moderate) (0.8)	Deleterious (supporting) (0)	Deleterious (Moderate) (1)	Deleterious (1)	Deleterious (1)	Benign (moderate) (0.15)	Deleterious (1)	Deleterious (0.73)
KMT2C: c.13174C>T	Uncertain (0.57)	Uncertain (0.018)	(N/A)	Deleterious (1)	Deleterious (0.99)	Uncertain (0.02)	Deleterious (1)	Deleterious (0.71)

[Table/Fig-3]: Prediction score from eight different tools.



[Table/Fig-4]: Case 1 – Rett Syndrome. General observations include lack of eye contact and abnormal hand movements.

Neurological examination revealed GDD [Table/Fig-1,5a,g]. Clinical observations suggested a genetic abnormality, but karyotyping was normal. WES identified the variant c.753\_754delCT (p.Val253Alafs\*44) in exon 7 of CHRNG.

**Case 3:** A six-year-old was identified with developmental delay, hyperactivity, behavioural issues, microcephaly, inadequate socio-adaptive functioning, ID, mild autism, epicanthal folds, a long philtrum, supernumerary nipples, hypospadias, kyphosis, pes planus, and congenital radioulnar synostosis [Table/Fig-1]. He was clinically suspected of having Costello syndrome/Pallister-Killian syndrome/3-M syndrome. WES identified a pathogenic c.785G>A (p.Arg262Gln) variant in exon 3 of GNRHR, associated with hypogonadotropic hypogonadism, with or without anosmia.

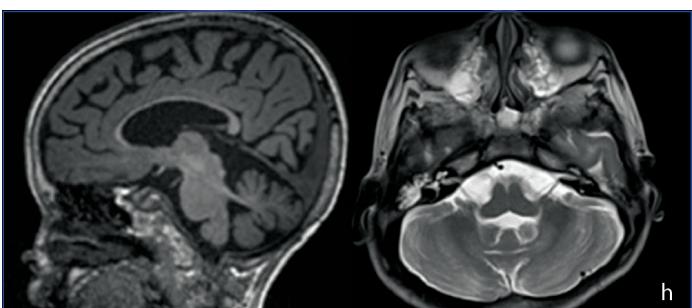
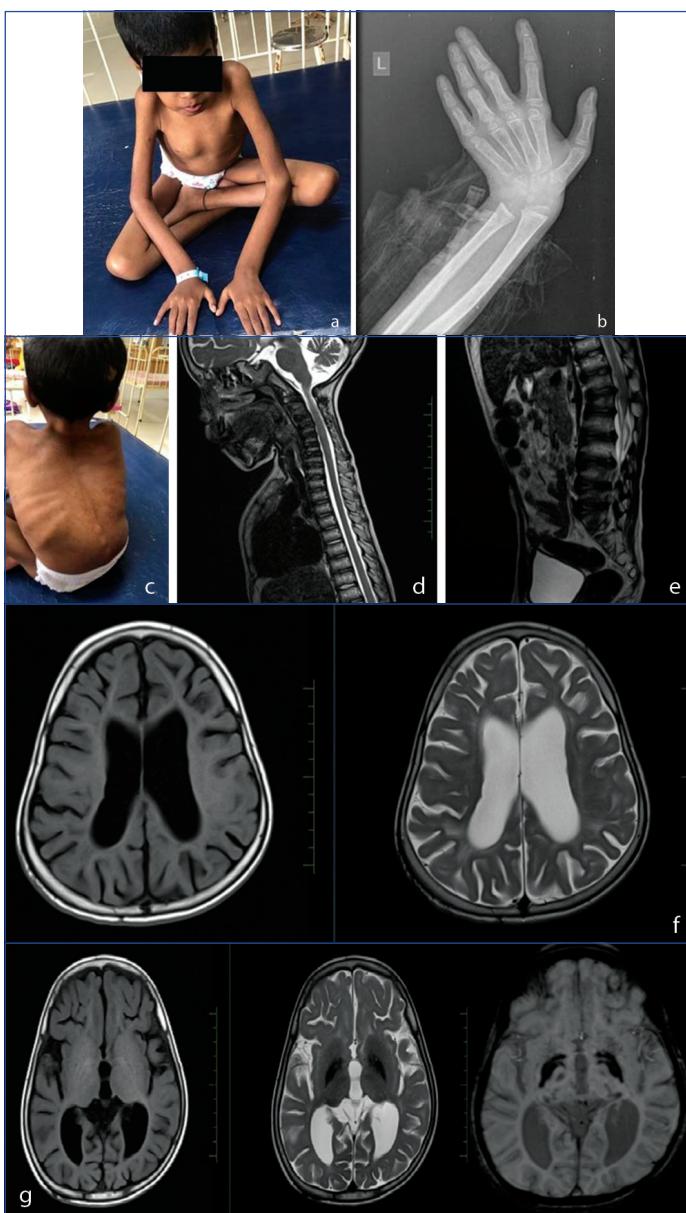
**Case 4:** A 14-year-old presented with developmental delay, congenital heart disease, renal tubular acidosis, short stature, coarse facies, dolicocephaly head and normal eyes. The patient was suspected of having mucopolysaccharidosis [Table/Fig-1,6a,h]. WES identified an autosomal homozygous stop-gained mutation (c.262C>T, p.Gln88Ter) in exon 1 of the FUCA1 gene.

**Case 5:** A 10-year-old exhibited diffuse encephalopathy and abnormal epileptic discharge in the right temporal region [Table/Fig-1]. WES identified two heterozygous mutations in AKT3, c.1393C>T (p.Arg465Trp), and PTCH1, c.169delA (p.Arg57Glyfs\*22).

**Case 6:** A seven-month-old baby presented with developmental delay and low bilateral plantar reflex [Table/Fig-1]. In this patient, three heterozygous missense variants were identified in the ATAD3A gene, c.526C>T (p.Arg176Trp), the TTRAP gene, c.3257G>A (p.Gly1086Glu), and the ATN1 gene, c.3057G>C (p.Glu1019Asp).



**[Table/Fig-5]:** Clinical phenotype of Case 2. Major features of Escobar syndrome include multiple contractures (arthrogryposis) and multiple pterygia. Shown are hand contractures (a), rocker-bottom feet with prominent heels (b,c), and pterygia of the elbow and knee with dimpling (d-f). The general appearance includes a V-shaped mouth with downturned corners (g).



**[Table/Fig-6]:** Clinical phenotype of Case 4. Skeletal abnormalities are shown in panels a and b. Biconcave-shaped vertebrae are observed from T12 to L3 (c,d), and platyspondyly with anterior beaking is noted from L3 to L5 (e). MRI reveals diffuse T2/FLAIR hyperintensities in the bilateral periventricular white matter and bilateral fronto-temporo-parietal subcortical white matter, suggestive of white matter leukoencephalopathy (f). Marked hypointensities are seen in the bilateral globus pallidus (g), substantia nigra, and red nuclei (h), showing blooming on the magnitude sequence of SWI and appearing hyperintense.

**Case 7:** A four-year-old presented with recurrent fever, failure to gain weight, constipation, dental caries and dysmorphic features, including a triangular face, low-set ears and retrognathia [Table/Fig-1]. Two novel heterozygous variants were identified in the RIPK1 gene, c.1808G>A (p.Arg603His), and the KMT2C gene, c.13174C>T (p.Pro4392Ser) [Table/Fig-2].

All patients received symptomatic management and were regularly monitored by a paediatric neurologist.

## DISCUSSION

WES is applied for global screening of mutations or polymorphisms that cause monogenic, oligogenic and multifactorial diseases. In individuals with severe developmental delays, microcephaly, seizures, dysmorphic facial features and poor muscle mass, 29,860 variants in 19,160 genes have been studied using WES [19]. In this study, WES was conducted in seven patients presenting with GDD to identify the specific variants associated with their phenotypes. The individual cases are discussed below.

Case 1 had a c.916 C>T (p.Arg306Cys) mutation in exon 4 of the MeCP2 gene. Although over 200 different genetic changes in MECP2 have been associated with Rett syndrome (RTT) (RettBASE: <http://mecp2.chw.edu.au>), almost 70% of all sporadic mutations found in

typical RTT arise from C-T transitions at mutation hot spots in the methyl DNA-binding domain (MBD) and NCoR/SMRT Interaction Domain (NID) [20,21]. Among these, Arg306Cys is one of the most common missense mutations associated with RTT syndrome [22]. It is located within the NCoR/SMRT Interaction Domain (NID) and inhibits transcriptional repression by disrupting the NCoR/SMRT interaction [23].

In Case 2, a frameshift mutation in exon 7 of the CHRNG gene: c.753\_754delCT was identified, which results in the premature truncation of the gamma subunit protein (p.Val253Alafs\*44). This variant leads to a frameshift starting at codon 253, introducing a premature stop codon 44 amino acids downstream. Such a truncation is predicted to severely disrupt the structure and function of the gamma subunit of the nicotinic acetylcholine receptor (AChR), which plays a crucial role in foetal neuromuscular junction formation. This particular mutation has previously been reported in Turkish families, where it was associated with the Lethal Multiple Pterygium Syndrome (LMPS) phenotype [24,25]. Recently, Manjunathan S et al., reported this same variant in an Indian patient, thereby expanding the known geographic and ethnic distribution of this pathogenic mutation [26]. The clinical features in present case align with those described previously, suggesting highly deleterious and likely uniform phenotypic effects across populations.

In Case 3, the variant reported in the GNRHR gene, specifically c.785G>A (p.Arg262Gln), is associated with Hypogonadotropic hypogonadism 7. This missense variant reduces signal transduction and is functionally deficient [27]. However, in present case, the clinical phenotype extends beyond the classical presentation of Hypogonadotropic hypogonadism 7. The patient also exhibited a broad spectrum of neurodevelopmental and dysmorphic features, suggesting a complex and syndromic presentation. Further genetic evaluation may be warranted to rule out additional contributory variants, such as deep intronic mutations that are not captured by standard WES.

In Case 4, WES identified an autosomal homozygous, stop-gained mutation (c.262C>T, p.Gln88Ter) in exon 1 of the FUCA1 gene that may result in protein truncation. To our knowledge, this variant has not been previously reported as pathogenic or benign. The known pathogenic variants in the FUCA1 gene are associated with fucosidosis [28]; eight of these loss-of-function variants are downstream of c.262C>T, emphasising the importance of this region for the proper function of the protein. The clinical observations in this case align with fucosidosis, albeit with a novel variant.

In Case 5 [Table/Fig-7a,b], two heterozygous mutations were observed in the AKT3 and PTCH1 genes. A variant in the AKT3 gene, c.1393C>T (p.Arg465Trp), has been previously associated with megalencephaly and polymicrogyria [29]. The other variant is a novel loss-of-function mutation in the PTCH1 gene, c.169delA (p.Arg57Glyfs\*22). Heterozygous mutations in PTCH1 are known to cause Holoprosencephaly-7 (HPE7) in humans [30]. Although the clinical features of this patient did not completely align with the observed variants, it is possible that these variants together

may contribute to epileptic episodes the patient did not have any megalencephaly and polydactyly which was atypical of the presentation related to AKT3 gene pathogenic mutation.

Case 6 was reported to harbour three heterozygous missense mutations in the ATAD3A, TRRAP, and ATN1 genes. In previous studies, these genes have been implicated in neurodevelopmental disorders. The variant in ATAD3A, c.526C>T (p.Arg176Trp), has been observed at a low frequency (0.0033%) in individuals of Asian descent in the gnomAD database [31]. This low frequency indicates that the variant is unlikely to be a common benign polymorphism; however, it is not linked to a known disease phenotype in large population databases. The other two variants, TRRAP c.3257G>A (p.Gly1086Glu) and ATN1 c.3057G>C (p.Glu1019Asp), are novel. As a result, it is not possible to definitively determine whether they play a causal role in the patient's neurodevelopmental delay.

In Case 7, two novel heterozygous variants were identified in the RIPK1 and KMT2C genes. A homozygous mutation in the RIPK1 gene is known to cause Immunodeficiency-57 with autoinflammation (IMD57), which aligns with the patient's symptoms [32]. However, in this study, authors observed a heterozygous mutation in RIPK1, complicating the interpretation. The dysmorphic features in this patient are consistent with Kleefstra syndrome-2 (KLEFS2), which is associated with a previously identified heterozygous mutation in the KMT2C gene [33]. Although the observed variant has the potential to contribute to the disease, a definitive diagnosis cannot be made based on these findings alone.

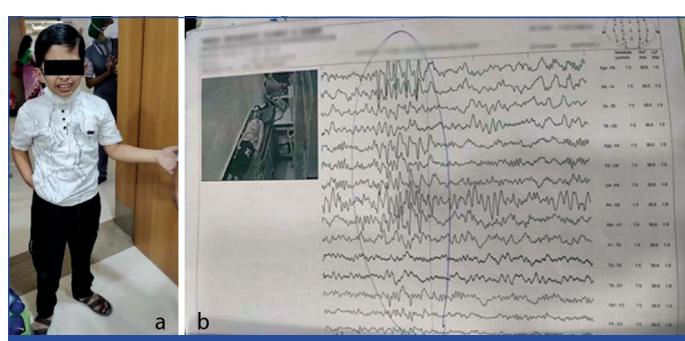
## CONCLUSION(S)

WES could identify the underlying genetic causes for patients with unexplained developmental delay DD/ID. Present series findings not only broaden the known mutation spectrum of genes associated with DD/ID but also highlight the potential of WES analysis in clinical diagnosis and the discovery of disease-causing mutations.

**Ethics statement:** The studies involving human participants were reviewed and approved by the IEC of the Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune, Maharashtra, India. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minors' legal guardian/next of kin, for the publication of any potentially identifiable data included in this article.

## REFERENCES

- Wondmagegn T, Girma B, Habtemariam Y. Prevalence and determinants of developmental delay among children in low-and middle-income countries: A systematic review and meta-analysis. *Frontiers in Public Health*. 2024;12:1301524. Doi: 10.3389/fpubh.2024.1301524.
- Juneja M, Gupta A, Sairam S, Jain R, Sharma M, Thadani A, et al. Diagnosis and Management of Global Development Delay: Consensus Guidelines of Growth, Development and Behavioral Pediatrics Chapter, Neurology Chapter and Neurodevelopment Pediatrics Chapter of the Indian Academy of Pediatrics. *Indian Pediatr*. 2022;59(5):401-15.
- Lakshmi NA, Deshmukh PR, Tripathy JP, Dahake U. Prevalence and determinants of developmental delay in children of 12–36 months in the area of primary health centre, Bela, Nagpur. *Indian J Public Health*. 2024;68(3):355-61. Doi: 10.4103/ijph.ijph\_334\_23.
- Wang T, Hoekzema K, Vecchio D, Wu H, Sulovari A, Coe BP, et al. Large-scale targeted sequencing identifies risk genes for neurodevelopmental disorders. *Nat Commun*. 2020;11(1):4932. Doi: 10.1038/s41467-020-18723-y.
- Shchubelka K, Turova L, Wolfsberger W, Kalanquin K, Williston K, Kurutsa O, et al. Genetic determinants of global developmental delay and intellectual disability in Ukrainian children. *J Neurodev Disord* [Internet]. 2024;16(1):13. Doi: 10.1186/s11689-024-09528-x.
- Han JY, Jang W, Park J, Kim M, Kim Y, Lee IG. Diagnostic approach with genetic tests for global developmental delay and/or intellectual disability: Single tertiary center experience. *Ann Hum Genet*. 2019;83(3):115-23. Doi: 10.1111/ahg.12294.
- Zanardo EA, Monteiro FP, Chehimi SN, Oliveira YG, Dias AT, Costa LA, et al. Application of whole-exome sequencing in detecting copy number variants in patients with developmental delay and/or multiple congenital malformations. *J Mol Diagn*. 2020;22(8):1041-49. Doi: 10.1016/j.jmoldx.2020.05.007.



**Table/Fig-7:** Clinical phenotype of case 5 macrocephaly with abnormal EEG slowing of activity suggestive of diffuse encephalopathy and abnormal epileptic discharge at right temporal region (a,b).

[8] Srivastava S, Love-Nichols JA, Dies KA, Ledbetter DH, Martin CL, Chung WK, 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. Doi: 10.1038/s41436-019-0554-6.

[9] Xiang J, Ding Y, Yang F, Gao A, Zhang W, Tang H, et al. Genetic analysis of children with unexplained developmental delay and/or intellectual disability by whole-exome sequencing. *Front Genet.* 2-021;12:738561. Doi: 10.3389/fgene.2021.738561.

[10] Vissers LE, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. *Nat Rev Genet.* 2016;17(1):09-18. Doi: 10.1038/nrg3999.

[11] Foo YL, Chow JC, Lai MC, Tsai WH, Tung LC, Kuo MC, et al. Genetic evaluation of children with global developmental delay-current status of network systems in Taiwan. *Pediatr Neonatol.* 2015;56(4):213-19. Doi: 10.1016/j.pedneo.2014.08.001.

[12] Kendig KI, Baheti S, Bockol MA, Drucker TM, Hart SN, Hedenbrand JR, et al. Sentieon DNASeq variant calling workflow demonstrates strong computational performance and accuracy. *Front Genet.* 2019;10:736. Doi: 10.3389/fgene.2019.00736.

[13] Poplin R, Chang PC, Alexander D, Schwartz S, Colthurst T, Ku A, et al. A universal SNP and small-indel variant caller using deep neural networks. *Nat Biotechnol.* 2018;36:983-87. Doi: 10.1038/nbt.4235.

[14] Landrum MJ, Chitipiralla S, Brown GR, Chen C, Gu B, Hart J, et al. ClinVar: Improvements to accessing data. *Nucleic Acids Research.* 2020;48(D1):D835-D844. Doi: 10.1093/nar/gkz972.

[15] Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res.* 2014;42(D1):D1001-D1006. Doi: 10.1093/nar/gkt1229.

[16] 1000 Genomes Project Consortium; Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, et al. A global reference for human genetic variation. *Nature.* 2015;526(7571):68. Doi: 10.1038/nature15393.

[17] Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature.* 2020;581(7809):434-43. Doi: 10.1038/s41586-020-2308-7

[18] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, 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):405-23. Doi: 10.1038/gim.2015.30.

[19] Hegde M, Santani A, Mao R, Ferreira-Gonzalez A, Weck KE, Voelkerding KV. Development and validation of clinical whole-exome and whole-genome sequencing for detection of germline variants in inherited disease. *Arch Pathol Lab Microbiol.* 2017;141(6):798-805. Doi: 10.5858/arpa.2016-0622-RA.

[20] Wan M, Lee SS, Zhang X, Houwink-Manville I, Song HR, Amir RE, et al. Rett syndrome and beyond: Recurrent spontaneous and familial MECP2 mutations at CpG hotspots. *Am J Hum Genet.* 1999;65(6):1520-29. Doi: 10.1086/302690.

[21] Neul JL, Fang P, Barrish J, Lane J, Caeg EB, Smith EO, et al. Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. *Neurology.* 2008;70(16):1313-21. Doi: 10.1212/01.wnl.0000291011.54508.aa.

[22] Brown K, Selfridge J, Lagger S, Connelly J, De Sousa D, Kerr A, et al. The molecular basis of variable phenotypic severity among common missense mutations causing Rett syndrome. *Hum Mol Genet.* 2016;25(3):558-70. Doi: 10.1093/hmg/ddv496.

[23] Lyst MJ, Ekiert R, Ebert DH, Merusi C, Nowak J, Selfridge J, et al. Rett syndrome mutations abolish the interaction of MeCP2 with the NCoR/SMRT co-repressor. *Nat Neurosci.* 2013;16(7):898-902. Doi: 10.1038/nn.3434.

[24] Bayram Y, Karaca E, Akdemir ZC, Yilmaz EO, Tayfun GA, Aydin H, et al. Molecular etiology of arthrogryposis in multiple families of mostly Turkish origin. *J Clin Invest.* 2016;126(2):762-78. Doi: 10.1172/JCI84457.

[25] Morgan NV, Brueton LA, Cox P, Greally MT, Tolmie J, Pasha S, et al. Mutations in the embryonal subunit of the acetylcholine receptor (CHRNG) cause lethal and Escobar variants of multiple pterygium syndrome. *Am J Human Genet.* 2006;79(2):390-95. Doi: 10.1086/506256.

[26] Manjunathan S, Singh K, Saini L. Multiple pterygium syndrome (Escobar Syndrome) a rare form of prenatal myasthenia presenting with arthrogryposis multiplex congenita. *Neurology.* 2024;103(2):e209602. Doi: 10.1212/WNL.0000000000209602.

[27] Šmigoc Schweiger D, Davidović Povše M, Trebušak Podkrajšek K, Battelino T, Avbelj Stefanija M. GNRHR-related central hypogonadism with spontaneous recovery - case report. *Ital J Pediatr.* 2022;48(1):184. Doi: 10.1186/s13052-022-01377-5.

[28] Stepien KM, Ciara E, Jezela-Stanek A. Fucosidosis- Clinical manifestation, long-term outcomes, and genetic profile- Review and case series. *Genes.* 2020;11(11):1383. Doi: 10.3390/genes1111383.

[29] Alcantara D, Timms AE, Gripp K, Baker L, Park K, Collins S, et al. Mutations of AKT3 are associated with a wide spectrum of developmental disorders including extreme megalencephaly. *Brain.* 2017;140(10):2610-22. Doi: 10.1093/brain/awx203.

[30] Ming JE, Kaupas ME, Roessler E, Brunner HG, Golabi M, Tekin M, et al. Mutations in PATCHED-1, the receptor for SONIC HEDGEHOG, are associated with holoprosencephaly. *Hum Genet.* 2002;110(4):297-301. Doi: 10.1007/s00439-002-0695-5.

[31] Chen S, Francioli LC, Goodrich JK, Collins RL, Kanai M, Wang Q, et al. Author correction: A genomic mutational constraint map using variation in 76,156 human genomes. *Nature.* 2024;626(7997). Doi: 10.1038/s41586-024-07050-7.

[32] Zhang J, Jin T, Aksentijevich I, Zhou Q. RIPK1-associated inborn errors of innate immunity. *Front Immunol.* 2021;12:676946. Doi: 10.3389/fimmu.2021.676946.

[33] Siano MA, De Maggio I, Petillo R, Coccidiiferro D, Agolini E, Majolo M, et al. De novo mutation in KMT2C manifesting as Kleefstra syndrome 2: Case report and literature review. *Pediatr Rep.* 2022;14(1):131-39. Doi: 10.3390/pediatric14010019.

#### PARTICULARS OF CONTRIBUTORS:

- Associate Professor, Department of Paediatrics, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pune, Maharashtra, India.
- Resident, Department of Paediatrics, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pune, Maharashtra, India.
- Scientist, Department of Central Research, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pune, Maharashtra, India.
- Consultant Geneticist, Department of Paediatrics, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pune, Maharashtra, India.
- Professor and Head, Department of Paediatrics, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pune, Maharashtra, India.

#### NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Renuka Majjigudda,  
Resident, Department of Paediatrics, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pune, Maharashtra, India.  
E-mail: renukamajjigudda232@gmail.com

#### AUTHOR DECLARATION:

- Financial or Other Competing Interests: Supported by Dr. D. Y. Patil Vidyapeeth, Pune
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

#### PLAGIARISM CHECKING METHODS: [\[Jan H et al.\]](#)

- Plagiarism X-checker: Mar 03, 2025
- Manual Googling: Apr 24, 2025
- iThenticate Software: May 10, 2025 (8%)

#### ETYMOLOGY: Author Origin

EMENDATIONS: 8

Date of Submission: **Feb 28, 2025**

Date of Peer Review: **Mar 23, 2025**

Date of Acceptance: **May 12, 2025**

Date of Publishing: **Jun 01, 2025**