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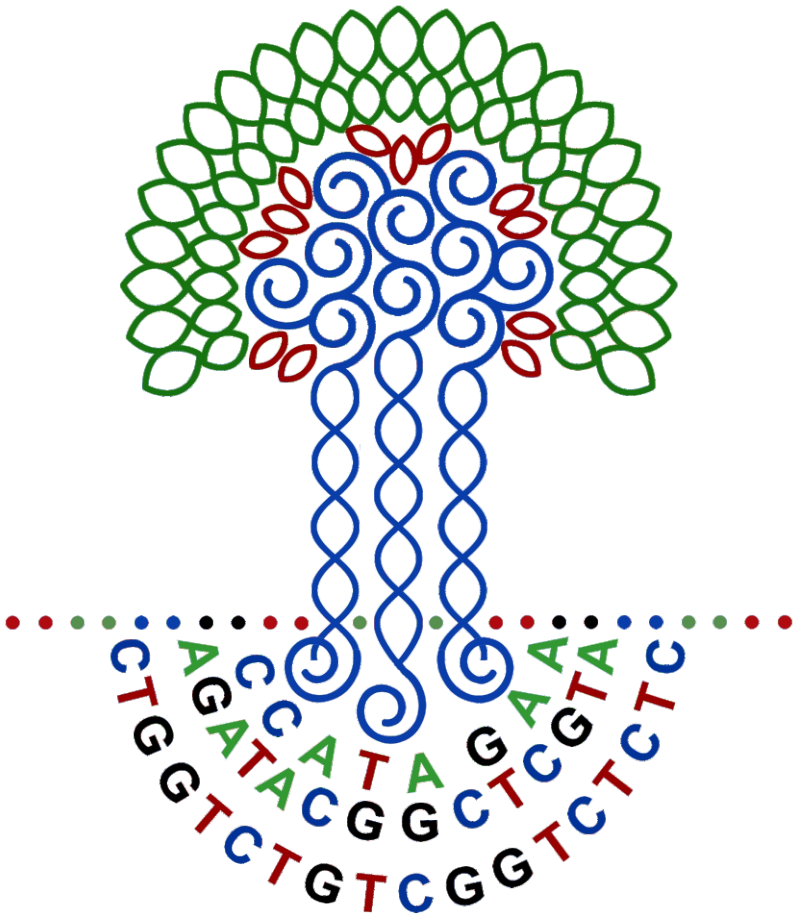

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PhotoQuiz - 66

Contributed by: Dr Haseena Sait

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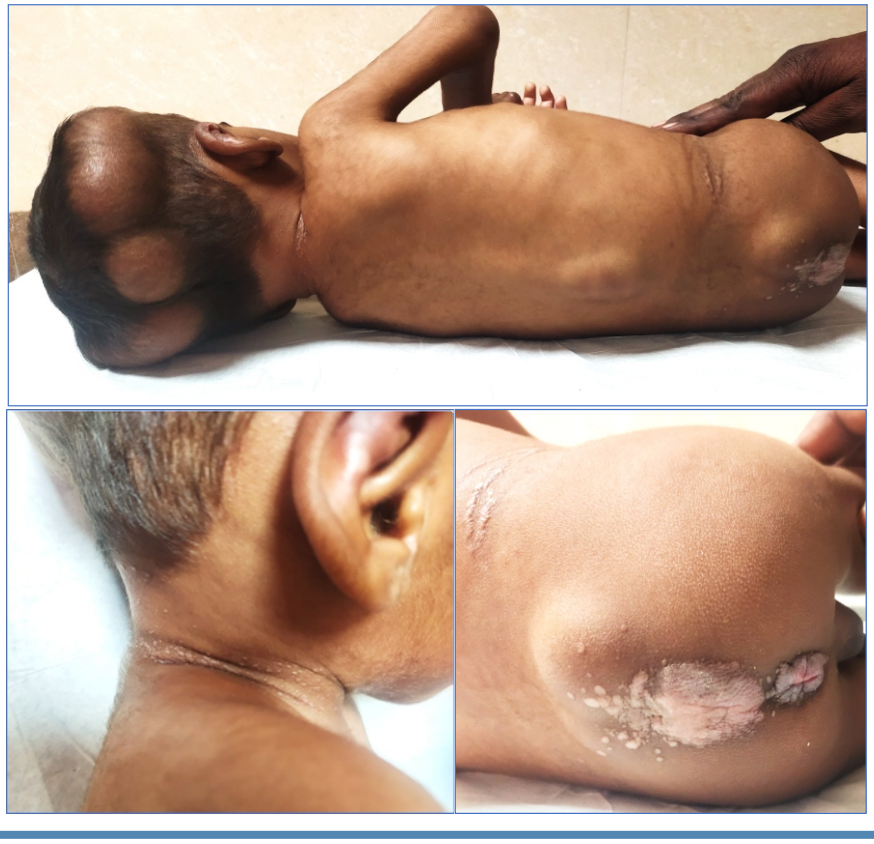
This 2-year-old male child, born of third-degree consanguineous marriage, presented with coarse facies, gingival hypertrophy, multiple nodular swellings over the scalp and lumbosacral region, pearly papules of the neck and perianal region, and contractures of the large and small joints. Identify the condition

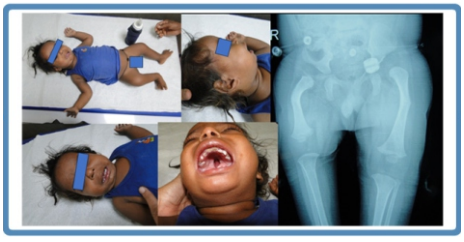
Please send your responses to editor@iamg.in
Or go to http://iamg.in/genetic_clinics/photoquiz_answers.php
to submit your answer.



Answer to PhotoQuiz 65

Femoral Hypoplasia – Unusual Facies Syndrome (OMIM %134780)

'Femoral Hypoplasia – Unusual Facies Syndrome' also known as 'Femoral – Facial Syndrome' is a rare disorder associated with bilateral femoral hypoplasia and dysmorphic facial features including upslanting palpebral fissures, a short nose with a broad tip, long philtrum, thin upper lip, micrognathia, and cleft palate. Renal anomalies and intracranial anomalies have also been reported in some affected individuals. The femoral involvement can be asymmetric. Majority of cases occur sporadically and do not have an identifiable etiology, but a similar phenotype can occur due to the teratogenic effects of maternal diabetes.



Correct responses to PhotoQuiz 65 were sent by:

1. Dr Vibha Jain. Anuvanshi, Ghaziabad, India.
2. Dr Beena Suresh. Mediscan System, Chennai, India.
3. Dr Karthick. Nottingham, United Kingdom.

GeNeDit

Genetics, Genomics and Clinical Geneticists: What's ahead?

Editorial

As one walks towards the horizon, distant pictures start becoming clearer while new blurred images appear on the horizon. We need to walk further to get a clearer view of them. Same thing happens in genetic diagnostics. The techniques to evaluate genes and the genome have improved greatly but one investigation for all genetic diagnostics keeps on evading the clinicians. More than three decades ago, clinical diagnosis had a lot of limitations as cases with signs and symptoms overlapping with two genetic disorders or novel phenotypes were a common experience. And young minds like me were waiting for identification of causative genes for all genetic disorders especially for malformation syndromes with the expectation that we shall be able to put cases in bins with the right diagnosis. As per current OMIM statistics, more than 7500 phenotypes with known causative genes and about 5000 genes with known phenotypes are documented. It is obvious that there are some genes with multiple phenotypes. About 1500 genes are associated with 2, 3, 4 or more phenotypes each. Also, we know many phenotypes are genetically heterogenous. Hence, one gene for one phenotype does not work either way!

Next generation sequencing has made diagnosis easy for most of the monogenic disorders and presentations with possible genetic diagnosis. Whole genome sequencing is taking care of intronic variants, copy number variations, structural variants and balanced rearrangements in one go. The technique of long read sequencing appears to be very powerful for novel types of variants including triplet repeats. It is especially helpful in identifying structural variants as exemplified by publications referred to in the GenExpress of this issue. One has been waiting for whole genome sequencing to get cheaper so that one investigation for every patient or genomic data in everyone's pocket even before symptoms appear will be the reality soon. It now seems that there is a choice of technology for sequencing the genome. Analyzing strategies also vary and may have different detection abilities.

As exome sequencing has made genetic diagnosis easy and there is awareness about presentations of genetic disorders amongst clinicians, many diagnoses are made in settings outside medical genetics departments. This is good in a way as not even developed countries have enough medical / clinical geneticists to take care of ever-expanding numbers of patients and families with genetic disorders. But it also calls for genomic education amongst non-genetics clinicians and complementary services of genetic counsellors. The judicious and cost-effective use of diagnostic techniques requires good knowledge of basic genetics, molecular genetics and principles of genomic testing and if there is lack of this there is a fear of over testing or missing a simple diagnosis. Interpretation of reports and communication of these high-end reports with lifelong implications to the patient and the family needs medical doctors with training in medical genetics. The increasing market of genomic diagnostics calls for urgent short-term, long-term, offline, and online courses in medical genetics for clinicians at various stages of their careers and incorporation of genomics in the undergraduate and postgraduate medical curriculum.

Genomics-related education is not only required to understand the power of genomics and genomic tools but also to know the limitations of these various technologies for high throughput sequencing. The power of knowing each nucleotide of the genome gives a bit of overconfidence about diagnosing all disorders pre-symptomatically in patients, and carrier screening for the purpose of reproductive decisions. Without clinical clues and suspicion of disorders with different disease mechanisms, the choice of exome sequencing and analysis of the data may be incorrect. The implications of results of uncertain significance, inability to predict phenotypes, non-penetrance, etc. are some of the many issues which need inputs of clinical/ medical geneticists for preventing harm to the individual,

as non-maleficence is the obligation of a clinician.

One more important role of clinical geneticists in genetic diagnostics is phenotyping including reverse phenotyping which has been highlighted in both the case reports in this issue. Now also, some clinical diagnoses are possible, especially with experienced clinical geneticists. If not possible, clinical phenotype-based classification and reverse phenotyping are very important parts of genetic diagnosis even in the era of high throughput sequencing and need an astute clinical geneticist. It is equally important for ordering the right test. In spite of all clinical expertise and access to advanced genetic tests still many cases remain undiagnosed. Research may identify novel genomic mechanisms unknown to date for disease pathogenesis and the search for one comprehensive test for every genetic disorder, without reliance on clinical data, will continue to be at the far away horizon. Genomics has to keep on marching ahead and clinical geneticists will have to play a major role in the second level of approach to diseases undiagnosed or misdiagnosed at the level of our non-geneticist clinical colleagues.

Dr. Shubha Phadke
1st October, 2024

Bohring-Opitz Syndrome: Report of a Patient with a Novel Variant in the *ASXL1* Gene & Review of Literature

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Abstract

Bohring-Opitz Syndrome (BOS) is a very rare genetic disorder with multiple anomalies caused by heterozygous pathogenic variants in the *ASXL1* gene. The child reported here had the classic presentation of BOS due to a novel pathogenic variant in the *ASXL1* gene.

Keywords: Bohring-Opitz syndrome, *ASXL1* gene

Introduction

Bohring-Opitz syndrome (BOS) is a very rare genetic disorder with multiple anomalies that primarily affects infants and young children and causes delay in growth and development, with involvement of various organ systems. This complex disorder presents with a wide spectrum of symptoms caused by *de novo* mutations in the *ASXL1* gene. The *ASXL* (additional sex comb-like) group of genes *(ASXL1, ASXL2, ASXL3)* play a critical role in the regulation of gene expression, particularly during development. They are involved in chromatin remodelling that are responsible for epigenetic and transcriptional regulation (Katoh, 2013) These genes have similar functions and variants in these genes present with overlapping clinical phenotypes Molecular diagnosis helps in delineating the various syndromes caused by the *ASXL* genes. Heterozygous pathogenic variants in the *ASXL1* gene are associated with the severe congenital-onset Bohring-Optiz syndrome, an autosomal dominant genetic disorder.

The child reported here had a classic presentation of BOS due a novel pathogenic variant in the *ASXL1* gene.

Clinical Description

This one-year-old female baby, the first offspring of a non-consanguineous couple, presented with low weight, global developmental delay (GDD), breathing difficulty, and failure to thrive. The antenatal history revealed that it was a spontaneous conception and the mother's antenatal sonograms were insignificant except for intrauterine growth restriction (IUGR) noted in the last trimester. During the antenatal period, the mother had severe anemia and hypothyroidism, requiring two blood transfusions and a daily dose of 100 micrograms of thyroxine tablets respectively. The baby was born full-term by normal vaginal delivery with a birth weight of 1.5 kg and cried immediately after birth. The Apgar score was 7/10 and 9/10 at one minute and 5 minutes respectively. The baby developed respiratory distress and required treatment in the neonatal intensive care unit (NICU) for 5 days. Starting from the 5th day, she was fed expressed breast milk. Due to poor weight gain and weak suckling efforts, her hospital stay was extended to 30 days. She remained on breast milk until 2 months of age, after which she was transitioned to formula feeding.

The baby had frequent respiratory tract infections, feeding difficulties, failure to thrive, and sleep disturbances. The developmental history revealed that the child had severe developmental delay and had not attained age-appropriate milestones, including gross motor skills such as neck control, rolling over, sitting, or standing. She had minimal speech development (only cooing), had not achieved social smile, and was not recognizing family members. Family history

Figure 1 Clinical findings in the patient. (A) Photograph of the patient showing typical BOS posture as described by Hastings et al., 2011. (B) Glabellar flammeus nevus, characterised by a distinctive port-wine stain marking on the forehead, typically associated with BOS, seen in the patient. (C) Oral inspection showing a high arched palate and an incomplete cleft. (D) Dermatological observation highlights deep palmar creases and a sacral dimple. (E) Neuroimaging through MRI showing posterior thinning of the corpus callosum indicative of atypical neural development associated with BOS.

revealed that one maternal uncle had died at 9 years of age with congenital skeletal anomalies and intellectual disability.

On examination, at one year of age, the baby's length was 58 cm (< - 3 Z score), weight was 3.5 kg (< -3 Z score), and head circumference was 39 cm (< -3 Z score). She had craniofacial dysmorphic features including scaphocephaly and microcephaly, bitemporal narrowing, long face, retrognathia, prominent forehead, proptosis, hypertelorism, arched eyebrows, up slanting palpebral fissures, glabellar flammeus nevus, depressed nasal bridge and low set ears. Oral examination revealed a cleft/notched lip, a deep and narrowed palate with a small mouth and unerupted deciduous dentition. On ophthalmic evaluation, she had large eyes with strabismus, ptosis in the left eye and mild myopia. She had the typical "BOS posture" with elbow and wrist flexion, camptodactyly, ulnar deviation of the wrists and metacarpophalangeal joints. Extremities were hypertonic with truncal hypotonia. Reverse phenotyping extended findings like deep palmar, plantar creases, and sacral dimples (**Figure 1**).

Magnetic resonance imaging (MRI) of the brain showed posterior thinning of the corpus callosum, mild dilatation of the trigone of both lateral ventricles and mild reduction in bilateral periventricular deep white matter volume with associated T2 FLAIR hyperintensities. Mutation analysis through whole exome sequencing revealed a novel heterozygous frameshift variant c.3125dup (p.Leu1043Thrfster7) in exon13 of the *ASXL1* gene [transcript ID NM_015338.6 (ENST00000375687.4 GRCH37/hg19 build)] Parental targeted testing was done through Sanger sequencing and both parents were negative for the variant (**Figure 2**). This indicates that this variant was most likely *de novo* in the proband. The variant is classified as 'likely pathogenic' (PVS1+PM2) according to the American College of Medical Genetics and Genomics & Association for Molecular Pathology (ACMG-AMP) guidelines. This established the diagnosis of Bohring-Opitz syndrome (OMIM # 605039) in the child.

Detailed genetic counselling was given to the parents outlining the reduced likelihood of another child inheriting the same disorder. The proband is being monitored regularly and

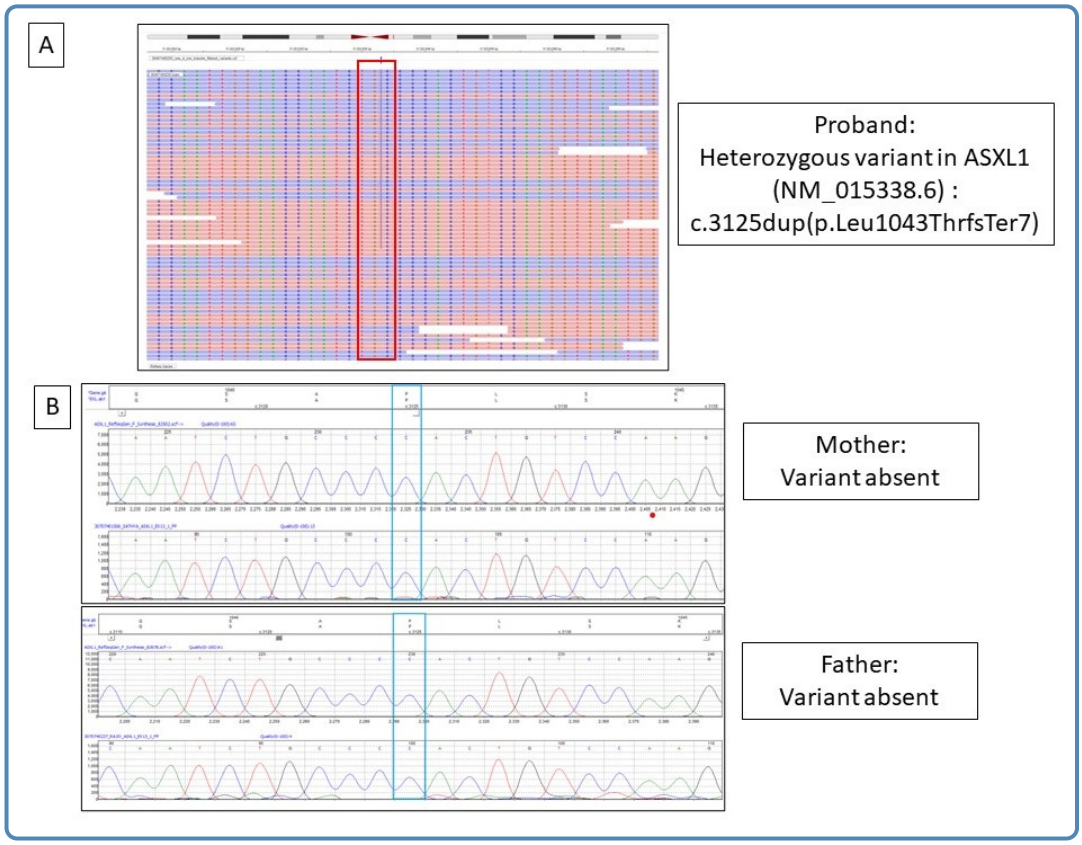

Figure 2 (A) Integrative Genome Viewer (IGV) image showing presence of the heterozygous variant c.3125dup (p.Leu1043Thrfster7) in exon13 of the *ASXL1* gene (ENST00000375687.4; GRCH37/hg19 build) in the proband. (B) Targeted variant testing in parents by Sanger sequencing showing absence of the variant in both parents.

ultrasonography of the abdomen once in four months up to eight years of age has been recommended as a part of renal tumour surveillance (Russell et al., 2023)

Discussion

Bohring - Opitz syndrome (BOS) was first described by the German paediatrician Helga V Bohring in 1999 and the American clinical geneticist John M Opitz in 2004. Bohring-Opitz Syndrome (BOS) is a rare genetic disorder characterised by a range of congenital anomalies and developmental delays caused by *de novo* mutations in the *ASXL1* gene. The ASXL group of genes plays a role in regulating tumour suppression and helps maintain the proper expression of genes necessary for cell differentiation and growth of the neural crest cells. Pathogenic variants in *ASXL2* and *ASXL3* are associated with Shashi-Pena and Bainbridge-Ropers syndrome, respectively (Cuddapah et al., 2021). All these syndromes exhibit overlapping clinical phenotypes like craniofacial dysmorphism, developmental delay, skeletal abnormalities and neurological manifestations (**Table 1**). Literature reports that *KLHL7* gene-associated Perching syndrome also exhibits BOS-like clinical features.

Clinical diagnostic criteria for BOS were given by Hastings et al. in 2011(Hastings et al., 2011). The typical clinical phenotype of BOS includes global developmental delay, failure to thrive, IUGR, microcephaly, craniofacial malformations, flammeus nevus, cleft lip/palate, retrognathia, prominent eyes with strabismus, and the typical "BOS posture" with flexion of the wrist and elbow, ulnar deviation of the metacarpophalangeal joints. As reviewed by Zhao and colleagues in 2021 (Zhao et al., 2021), 40 cases have been reported with *ASXL1* variants in literature. Mostly they occur

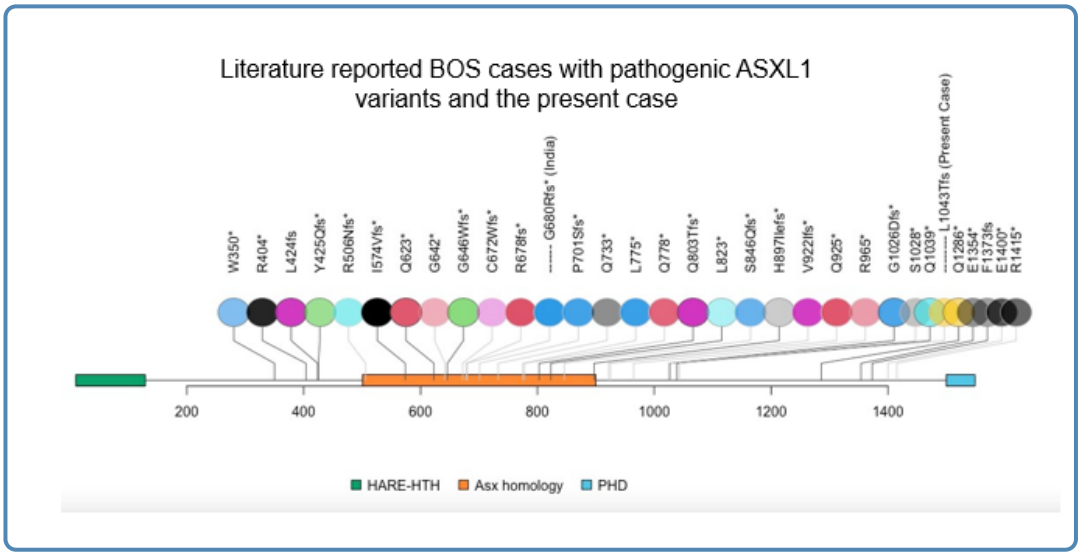

Figure 3 The schematic outlines the ASXL1 protein domain structure, which includes the HARE-HTH, ASX homology, and PHD domains, depicted in green, orange, and blue, respectively. The lollipop plot shows the *ASXL1* variants in the literature-reported cases of Bohring-Opitz Syndrome (Zhao et al. 2021; Russell et al., 2023) and highlights the variant identified in the present patient.

de novo with two cases in literature suggesting germline mosaicism so far (Greenhalgh et al.2003; Cuddapah et al. 2021). In India, among the cases that were reported as BOS, only one case was molecularly confirmed to the best of our knowledge (Arunachal et al., 2016).

In 2023, Russel and colleagues (Russell et al., 2023) described the clinical findings in 39 patients with BOS along with recommendations for tumour surveillance. They reported Wilms tumour in five patients and a novel finding of hepatoblastoma in one patient with BOS. They emphasised the importance of renal tumour surveillance in BOS patients every three to four months up to eight years of age (Russell et al., 2023) Though the somatic mutations in the *ASXL1* gene are associated with many haematological and solid tumours (Katoh, 2013; Micol & Abdel-Wahab, 2016) to date, other forms of cancers have not been reported with BOS except Wilms tumour and hepatoblastoma (Russell et al., 2023)

Here we have presented a case of Bohring-Opitz syndrome which fulfils the clinical criteria given by Hastings et al. (2011) and is molecularly confirmed with a heterozygous duplication variant in *ASXL1*. A genotype-phenotype correlation was established. Our variant has not been reported previously in any literature or databases like HGMD (https://www.hgmd.cf.ac.uk/), OMIM (https://www.omim.org/), UCSC Genome Browser (https://genome.ucsc.edu/), Ensembl (https://ensembl.org/index.html), dbSNP (`https://www.ncbi.nlm.nih.gov/snp/`) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). All the previously reported pathogenic variants in literature have been reviewed as a lollipop plot with the present case indicated in the disease-causing protein domain (**Figure 3**). We have submitted our variant to ClinVar (ID: 3338642 Accession ID: VCV003338642.1).

Due to the rarity and severity of Bohring-Opitz Syndrome and in view of the phenotypic heterogeneity of the *ASXL1* gene, clinical diagnosis of BOS and its management are highly challenging. Medical care typically focuses on managing the symptoms and providing supportive care to improve the quality of life for affected individuals. Thorough clinical examination with genotype-phenotype correlation helps in arriving at a confirmatory diagnosis in very rare genetic disorders like BOS.

Acknowledgements: We thank the parents of the proband who have granted permission for the reproduction of their daughter's details and photographs for this article.

Table 1 Clinical features differentiating the ASXL family of genes and their syndromes

Clinical Features	Bohring-Opitz syndrome *(ASXL1)*	Shashi Pena syndrome *(ASXL2)*	Bainbridge Ropers syndrome *(ASXL3)*
'BOS' posture	Y	N	N
Trigonocephaly	Y	N	N
Microcephaly	Y	N	Y
GDD	Y	N	Y
Prominent eyes	Y	N	Y
Ptosis	N	N	Y
Aggressive behaviours	N	N	Y
Hand flapping, rocking behaviours	N	N	Y
Macrocephaly	N	Y	N
Normal height & weight	N	Y	N
Epilepsy	Y	Y	N
Nevus flammeus	Y	Y	N
Precocious puberty	Y	N	N
Thick hair	Y	-	-
Arched eyebrows	Y	Y	Y
Hypertelorism	Y	Y	Y
Feeding difficulties	Y	Y	Y
Hypotonia	Y	Y	Y
Cognitive disabilities	Y	Y	Y

○ - Overlapping phenotypes, ○ - Specific for Bohring Opitz, ○ - Specific for Shashi Pena, ○ - Specific for Bainbridge Ropers. Y-Yes: N-No

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Clinical Vignette

Ehlers-Danlos Syndrome with Glycosaminoglycan Abnormalities: A Report of the Rare Musculocontractural and Spondylodysplastic Subtypes

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Abstract

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of genetic connective tissue disorders characterized by skin hyperextensibility, joint hypermobility, and atrophic scarring. The majority of cases are caused by mutations in the collagen-encoding or collagen-modifying genes. A rarer subset of EDS with atypical presentation is caused by an abnormality in glycosaminoglycan synthesis- the spondylodysplastic type of EDS related to mutations in the genes *B4GALT7, B3GALT6 or SLC39A13*, and the musculocontractural EDS caused by biallelic pathogenic variants in *CHST14* or *DSE* genes. We report two cases of EDS related to *CHST14* and *B3GALT6* gene variants and discuss the unique features of these rarer subtypes.

Keywords: Ehlers-Danlos, *B3GALT6* gene, *CHST14* gene, Spondylodysplastic EDS, Musculocontractural EDS

Introduction

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of genetic connective tissue disorders characterized by skin hyperextensibility, joint hypermobility, and atrophic scarring caused primarily by mutations in the collagen-encoding genes or genes encoding collagen-modifying enzymes. The 2017 International Classification of the Ehlers–Danlos Syndromes describes 13 types based on characteristic phenotypic manifestations (Malfait et al., 2017). The tenth subtype of EDS as per this nosology is caused by variations in genes coding for the enzymes responsible for adding sugar moieties to the linker region of proteoglycans and are also classified as "linkeropathies" (Caraffi et al., 2019). This subtype of EDS is called the spondylodysplastic EDS, previously classified as the progeroid EDS. Mutations in the genes *B4GALT7* and *B3GALT6* (coding for galactosyltransferase I and II respectively) and *SLC39A13* are described to be causative. Specific clinical diagnostic criteria have been defined, of which the major criteria comprise progressive short stature, hypotonia, and bowing of limbs. The eleventh described subtype called the musculocontractural Ehlers–Danlos Syndrome (MC-EDS) is caused by biallelic pathogenic variants in the gene for carbohydrate sulfotransferase 14/ dermatan 4-*O*-sulfotransferase 1 (*CHST14*/*D4ST1*) (MIM#601776), or the gene for dermatan sulfate epimerase (*DSE*) (MIM#615539) (Kosho et al., 2016; Brady et al., 2017). Major criteria for this subtype are multiple congenital joint contractures, characteristic facies, and prominent skin findings. Both the above types of EDS have common molecular abnormalities involving glycosaminoglycans which play an important role in the formation of collagen fibrils in the connective tissue. We describe one case each of musculocontractural EDS type 1 (*CHST14* associated) and spondylodysplastic EDS type 2 (*B3GALT6* associated), further reiterating the phenotypic spectrum of these conditions.

Patient description:

Patient 1: A 2-year-8-month-old female child, fifth born to third-degree consanguineous parents, was brought to the genetics clinic with bilateral clubfoot deformity since birth and motor developmental delay. She was born at term

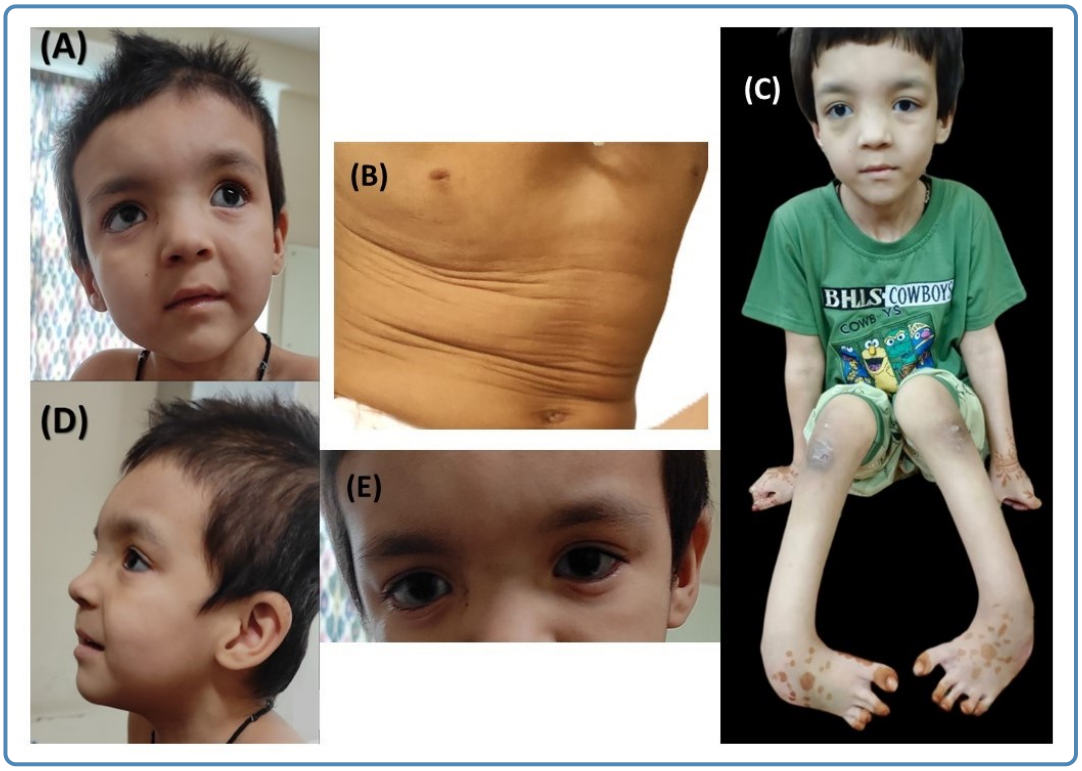

Figure 1 Child with EDS musculocontractural type 1. (A) Hypertelorism, epicanthal folds, down slanting palpable fissures, sagging skin on cheeks. (B) Excessive wrinkling & sagging of skin over the chest and abdomen with pectus excavatum. (C) Progeroid appearance, bilateral club feet, increased gap between 1st and 2nd toes, spatulate toes, callosity with scarring on the anterior aspect of the knee (D) Brachycephalic skull, low-set ears (E) Blue sclerae

with a birth weight of 3.5 kg. A significant motor delay was evident, and she had not achieved independent ambulation. Craniofacial dysmorphism was appreciated in the form of a brachycephalic skull, tall forehead, hypertelorism, down slanting palpebral fissure, epicanthal folds, blue sclera, broad nasal bridge, and long, smooth philtrum (**Figures 1A, 1D & 1E**). On head-to-toe examination, widely spaced nipples with sagging skin, increased skin fold over the trunk and abdomen, pectus excavatum (**Figure 1B**), and bilateral talipes equinovarus deformity were seen (**Figure 1C**). The skin had a soft doughy feel, was hyperextensible, and had excessive wrinkles. Shallow palmar creases were seen. Significant joint laxity and hypotonia were appreciated. The Z-scores for head circumference (-3.32), weight (-4.15), and height (-3.31) indicated significant failure to thrive. Other systemic examinations were normal. Her intellect and vision were normal. A provisional clinical diagnosis of cutis laxa or Ehlers-Danlos syndrome was made.

A skeletal survey showed scoliosis of the thoracolumbar spine and developmental dysplasia of bilateral hips. Hearing evaluation revealed bilateral profound hearing loss. Magnetic resonance imaging (MRI) of the brain showed T1 hypointensity and T2 hyperintensities in the subcortical region, involving the corpus callosum and corona radiata of the bilateral frontal regions (**Figure 3A**). 2D echocardiography of the heart and ophthalmological evaluation were within normal limits. Whole exome sequencing from peripheral leucocyte DNA revealed a homozygous missense variant in the *CHST14* gene (NM_130468.4) c.652C>A; p.Arg218Ser. Computational analysis with online tools such as Frankin by Genoox (`https://franklin.genoox.com/analysis-tool/join-cta`) and VarSome (`https://varsome.com/`) showed it was a likely pathogenic variant [criteria PP3 + PM2 + PP5 as per variant classification guidelines of the American College of Medical Genetics and Genomics & Association for Molecular Pathology (ACMG/AMP)]

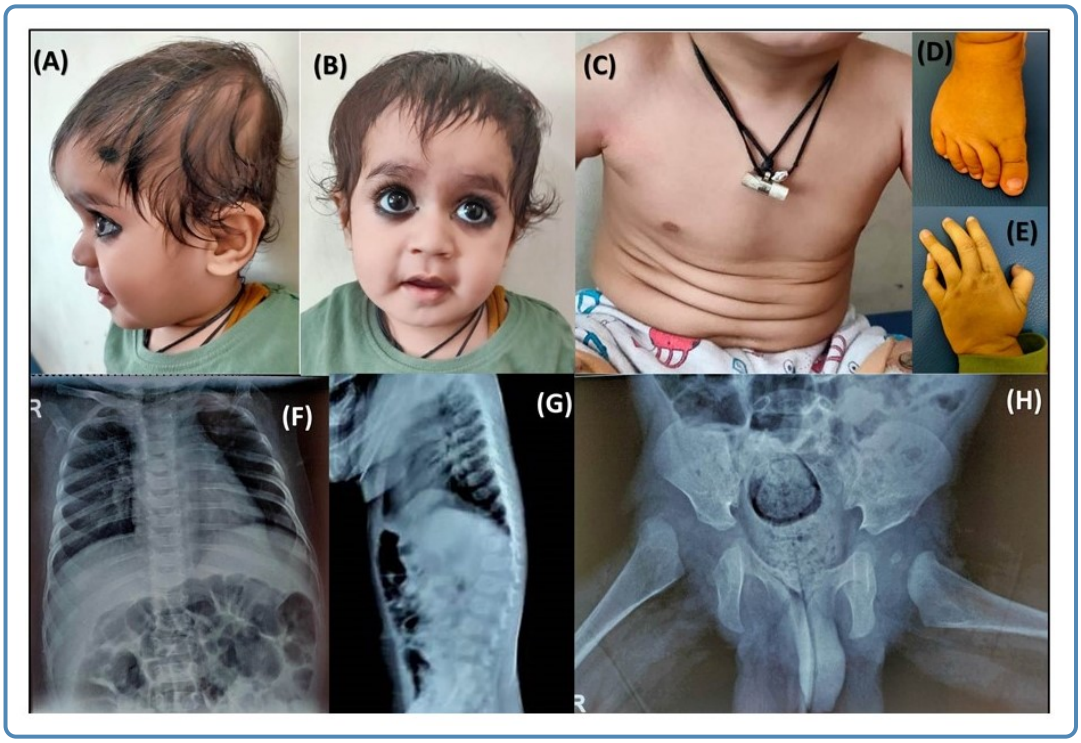

Figure 2 Child with EDS spondylodysplastic type 2. (A) Frontal bossing, deep-set eyes, long prominent philtrum, low-set prominent ears, sparse scalp hair. (B) Prominent eyes with mild down slant, and sagging cheeks. (C) Increased skin folds over the chest and abdomen. (D) & (E) Spatulate toes with radial deviation, long tapering fingers with ulnar deviation of third to fifth digit, and flexion contractures of proximal interphalangeal joints. (F) & (G) X-ray chest and spine showing exaggerated thoracic kyphosis with lumbar lordosis, unremarkable ribs, and vertebral bodies (H) X-ray pelvis showing bilateral hip dislocation.

and was consistent with the clinical phenotype. The patient was diagnosed to be affected with Ehlers-Danlos syndrome, MC-EDS type 1.

Patient 2: An 8-month-old boy, second born to a non-consanguineous couple, was brought with a history of motor developmental delay and developmental dysplasia of the hip (DDH). He was born at term with a weight of 3.5 kg. A plaster cast was applied for DDH at 4 months of age. On examination, he had craniofacial dysmorphisms such as frontal prominence, plagiocephaly, midface hypoplasia, deep-set prominent eyes with mild down slant, and long philtrum (**Figure 2A & 2B**). Sparse scalp hair (**Figure 2A**), and increased skin folds over the chest and abdomen were observed (**Figure 2C**). Increased joint laxity especially of hand and feet with a Beighton score of 6/9, and soft skin with increased palmar creases was appreciated. Spatulate toes with radial deviation, broad first toe, long tapering fingers with ulnar deviation of third to fifth digits, and flexion contractures of proximal interphalangeal joints were noted (**Figures 2D & 2E**). Hearing, vision, and cognition were normal. Length (67 cm) was on the 5th centile, head circumference (44 cm) on the 33rd centile, and weight (7 kg) on the 3rd centile. Central nervous system examination revealed hypotonia with diminished deep tendon reflexes. The pelvic radiograph showed bilateral hip dislocation (**Figure 2H**).

2D echocardiography detected a 3mm patent foramen ovale. Mixed hearing loss (unilateral) was present, and eye evaluation was normal. Radiographs showed generalized osteopenia with thin cortices, wavy long bones and ribs, thoracic kyphosis, and exaggerated lumbar lordosis (**Figures F&G**). A provisional diagnosis of Ehlers-Danlos syndrome or Larsen syndrome was made. Whole exome sequencing from leukocyte DNA revealed two variants in the *B3GALT6*

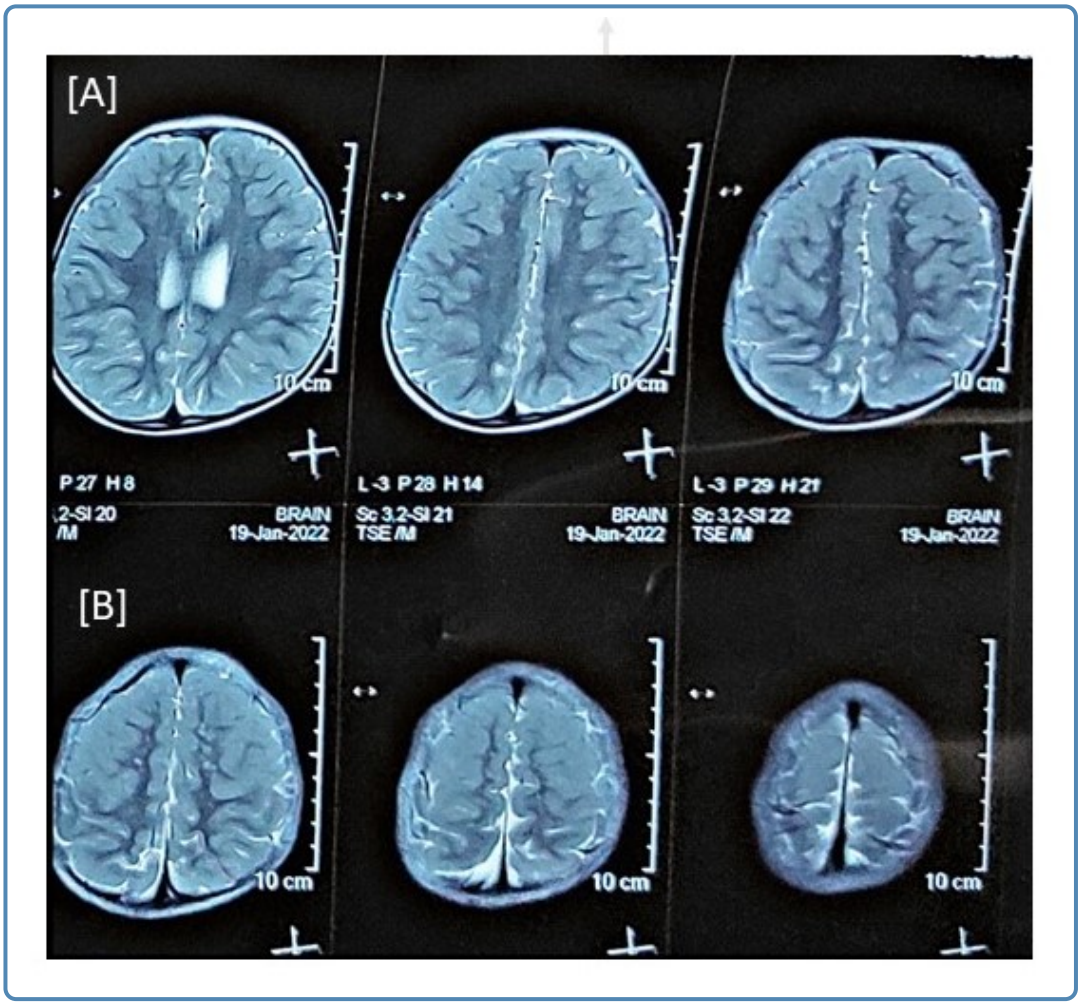

Figure 3 MRI images showing white matter changes in the child with MC-EDS [A] T2 hyperintensities in subcortical regions of bilateral frontal lobes [B] T2 hyperintensities in bilateral centrum semiovale.

gene, (NM_080605.4) c.545A>G; p.Tyr182Lys, a previously reported likely pathogenic variant (criteria PP3 + PM2 + PP2 + PP5 as per ACMG/ AMP guidelines) (Van Damme et al., 2018); and c.749C>T; p. Ala250Val, a novel variant of uncertain significance (criteria PM2 + PM1 + PP2 + PP3 as ACMG/ AMP guidelines). Both variants are not present in publicly available population databases (dbSNP - http://www.ncbi.nlm.nih.gov/SNP; 1000 Genomes - https://www.internationalgenome.org/; gnomAD - https://gnomad.broadinstitute.org/) *in-silico* prediction programs like SIFT (https://sift.bii.a-star.edu.sg/); PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/); MutationTaster2 (https://www.mutationtaster.org/); and CADD (https://cadd.bihealth.org/) predicted both these variants to be deleterious. Parental segregation analysis revealed each of the variants to be present in a heterozygous state in one of the parents. The clinical presentation and the molecular report indicated that the patient was affected with Spondylodysplastic Ehlers-Danlos syndrome type 2 (EDSSPD2).

Discussion

MC-EDS was reported to be caused by biallelic variants in the *CHST14* gene by Dündar et al. (2009). At present, 66 patients with 48 families are reported in the literature (Minatogawa et

al., 2022). Syx et al. (2015), described two patients from the same family, with homozygous c.652C>A variants in the *CHST14* gene, similar to our case. These patients presented with severe bilateral talipes equinovarus, dislocation of the hip but no adducted thumbs. Speech and motor development were delayed, in contrast to our case report where only motor developmental delay was evident. Very few Indian cases are reported (Lautrup et al., 2020). The clinical phenotype of our patient is consistent with the known phenotypic spectrum of this condition. However, our patient does not report any significant morbidity in the form of subcutaneous hematomas, recurrent joint dislocations, or systemic complications. This indicates possibly a mild phenotypic presentation or these findings could evolve. An unusual finding was the white matter changes in brain imaging. Previous reports have described periventricular heterotopias, ventricular enlargement/ asymmetry, absence of the left septum pellucidum, short corpus callosum, and cerebellar hypoplasia (Minatogawa et al., 2022). Mice models by Li et al. (2019) showed that Chst14/D4st1 deficiency resulted in impaired spatial learning, memory, and long-term potentiation. Chondroitin and dermatan sulfate are extracellular components of the central nervous system (CNS) and interact with growth factors and neurotrophic factors influencing neuronal migration, axon guidance, neurite outgrowth, and synaptic plasticity. The CNS findings in our case were likely a part of the EDS phenotype, and follow-up has been advised.

Mutations in *B3GALT6* were first described by Nakajima et al. (2013). Subsequently, Damme et al. (2018), described 12 cases of biallelic *B3GALT6* gene mutations causing EDSSPD. In recent literature, a heterozygous variant in *B3GALT6* co-segregated with clinical features such as elbow contracture, scoliosis, and facial dysmorphism (Shen et al., 2022). All patients with homozygous variants had features of both EDS and spondylo-meta-epiphyseal dysplasia. Some of them had complications like aortic dilatation/ aneurysm, cervical spine instability, and respiratory insufficiency. *B3GALT6* mutation causes complete loss of galactosyltransferase activity leading to deficient GAG synthesis and disrupted collagen organization. A patient with the variant (c.545A>G) in a homozygous state was described in a 3-year-old Iranian boy born of a consanguineous couple. Facial features were similar in both cases like frontal bossing, midface hypoplasia, downward slanting eyes, and long prominent philtrum. As of today, disease-causing variations in the *B3GALT6* gene have been reported in around 50 patients. The facial dysmorphic features, skin findings, and joint abnormalities in the previously reported patients are similar to the findings in our case. Previous studies have shown a significant burden of skeletal abnormalities including spinal deformities, fractures, and short stature, which were not present in our patient. These may be age-dependent features. Regular follow-up and serial radiographs are planned for the patient to look for these evolving findings. **Tables 1 & 2** depict the diagnostic major and minor criteria for these rare subtypes of EDS as per the nosology along with the features as seen in these two cases. Although hearing loss is not included in the major or minor criteria, both of our patients presented with sensorineural hearing loss, and similar cases have been reported in literature associated with both these subtypes of EDS.

These two patients reiterate the phenotypic presentation of two relatively rarer subtypes of EDS syndrome which involve abnormalities of the glycosaminoglycans (GAGs). GAGs are important components of connective tissue and help in the formation of the collagen fibril network. Disorders of their synthesis and processing result in complex forms of EDS which present with a more complicated clinical phenotype as compared to the EDS resulting from collagen mutations. This phenotype includes significant skeletal abnormality as seen in *B3GALT6*-associated EDSSPD, joint contractures as seen in *CHST14*-associated MC-EDS, as well as more widespread systemic involvement in the form of structural cardiac defects, brain abnormalities, ophthalmic, and hearing abnormalities, etc. This indicates the ubiquitous role of GAGs during embryonic development as well as a role in connective tissue integrity throughout the lifespan of the individual.

Conclusion

EDS resulting from glycosaminoglycan abnormalities are rarer, show severe phenotypes with multi-systemic involvement, and overlap with other conditions like skeletal dysplasia and arthrogryposis. Deep phenotyping helps in the computational analysis of exome sequencing data sets, enabling timely and accurate molecular diagnosis. This is crucial for adequate managem-

TABLE 1	MUSCULOCONTRACTURAL EDS	PREVALENCE OF FINDING	PATIENT 1
MAJOR CRITERIA	1. Congenital multiple contractures, characteristic adduction-flexion contractures and/or talipes equinovarus (clubfoot)	98%, 95%	+
	2. Characteristic craniofacial features, evident at birth/early infancy	Hypertelorism 92% Down-slanting palpebral fissures 95% Smallmouth 88% Blue sclerae 86% slender face 83% Long philtrum 80% Low-set ears 71% Thin upper lip 65% Brachycephaly 54% Midface hypoplasia 58%	+ + + + + + + + + +
	3. Cutaneous features- skin hyperextensibility, easy bruisability, skin fragility with atrophic scars increased palmar wrinkling	100%	+
MINOR CRITERIA	1. Recurrent/chronic dislocations	90%	
	2. Pectus deformities (flat, excavated)	84%	+
	3. Spinal deformities (scoliosis, kyphoscoliosis)	87%	+
	4. Peculiar fingers (tapering, slender, cylindrical)	87%	+
	5. Progressive talipes deformities (valgus, planus, cavum)	98%	+
	6. Large subcutaneous hematomas	81%	
	7. Chronic constipation	85%	
	8. Colonic diverticula	35%	
	9. Pneumothorax	10%	
	10. Nephrolithiasis	29%	
	11. Hydronephrosis	51%	
	12. Cryptorchidism in males	88%	
	13. Strabismus	66%	+
	14. Refractory errors	93%	
	15. Glaucoma/elevated IOP	49%	
	MINIMUM CRITERIA FOR CLINICAL DIAGNOSIS	1. At birth/early childhood- major criteria 1 and 2 2. In adolescence and adulthood- major criteria 1 and 3	3 major and 5 minor criteria

TABLE 2	SPONDYLODYSPLASTIC EDS	PREVALENCE OF FINDING	PATIENT 2
MAJOR CRITERIA	1. Short stature (progressive in childhood)	99%	+
	2. Muscle hypotonia	65%	+
	3. Bowing of limbs	80%	
MINOR CRITERIA	1. Skin hyperextensibility, soft doughy skin, thin translucent skin	85%	+
	2. Pes planus	75%	
	3. Delayed motor development		+
	4. Osteopenia	85%	+
	5. Delayed cognitive development	50%	
GENE-SPECIFIC MINOR CRITERIA (*B3GALT6*)	1. Kyphoscoliosis (congenital, early onset, progressive)	90%	
	2. Joint hypermobility	92%	+
	3. Joint contractures (congenital or progressive) especially hands	60%	+
	4. Peculiar fingers (slender, tapered, arachnodactyly, spatulate, with broad distal phalanges	80%	+
	5. Talipes equinovarus	62%	
	6. Characteristic craniofacial features	Plagiocephaly, prominent forehead 90% Asymmetric face & flat midface 75% Straight and fine hair 45% Sparse eyelashes Blue sclerae 70% Proptosis 60% Down-slanting palpebral fissures, High and narrow palate, malocclusion, low-set ears	 + + + + + +
	7. Tooth discoloration, dysplastic teeth	75%	
CHARACTERISTIC RADIOGRAPHIC FINDINGS	1. Osteoporosis with multiple spontaneous fractures		
	2. Ascending aorta aneurysm		
	3. Lung hypoplasia, restrictive lung disease		
CLINICAL DIAGNOSIS	1 and 2 of the major criteria plus characteristic radiographic abnormalities and at least 3 minor criteria		2 major and 7 minor criteria

ent, follow-up, and prevention of complications, to predict recurrence risk in the family, and for timely prenatal invasive testing. Quality of life can be improved with proper rehabilitation. Diligent follow-up of cases helps in preventing life-threatening complications or early death/ disability due to ruptured or dissecting aneurysm, massive hematomas, poor wound healing/infection, retinal detachment, fractures, joint dislocation followed by vascular or neural compromise.

Conflict of Interests: None

Declaration: Informed consent was taken for clinical photographs from the guardian.

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Genetics of Neonatal Diabetes: An Update

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Abstract

Neonatal diabetes mellitus (NDM) is defined as persistent hyperglycemia in infants within the first six months of life or rarely within one year of life, along with absent or insufficient circulating insulin. It can be either transient or permanent diabetes, depending on the duration of insulin requirement. Transient NDM is frequently associated with aberrations of 6q24 locus resulting in overexpression of imprinted genes. Other pathogenic variations which cause transient NDM are activating mutations of K_{ATP} channel-related genes (*ABCC8* and *KCNJ11*), and insulin (*INS*) and *ZFP57* gene mutations. Permanent NDM is caused by a wide range of pathogenic variants causing beta cell functional defects, endocrine pancreas development abnormality, and beta cell destruction which is either immune mediated or secondary to endoplasmic reticulum stress. Most common pathogenic variants associated with permanent NDM are *KCNJ11*, *ABCC8*, *INS*, *GCK* and *PDX1* mutations. Several syndromic variants with extra-pancreatic features are also described to cause neonatal diabetes. Molecular genetic testing is important for prognostication, to decide on the appropriate therapeutic option (insulin or sulfonylureas) and for genetic counselling.

Keywords: Genetic basis, neonatal diabetes mellitus

Introduction

Hyperglycemia is one of the common metabolic disturbances seen in neonates, especially in the preterm, low-birthweight or very sick infants. To define as neonatal diabetes mellitus (NDM), there must be persistent hyperglycemia (plasma glucose >150 mg/dL) within the first six months or rarely within one year of life along with absent or insufficient circulating insulin (Beltrand et al., 2020). The global incidence varies from 1 in 90,000 to 160,000 live births (Lemelman et al., 2019). More than 50% of the cases are transient NDM (TNDM), in which hyperglycaemia usually resolves within 18 months (Polak et al., 2007). The remaining are permanent NDM which requires lifelong insulin therapy. The basic mechanism is either an abnormal development of pancreatic beta cells or a functional abnormality in insulin secretion. Consequently, neonatal diabetes may also be associated with pancreatic and extra-pancreatic malformations. It is a predominantly monogenic disease with 80% having an identified genetic mutation (Polak et al., 2007). Apart from conventional insulin replacement therapy, specific genetic mutations also respond to oral sulfonylureas. Hence, the role of genetic testing in NDM is crucial.

Molecular Pathology and Genetic Basis

Neonatal DM is broadly classified into **transient** or **permanent,** based on the duration of insulin requirement. **Syndromic NDM** can be either transient or permanent, associated with extra-pancreatic features that are part of a known inherited syndrome.

A) Transient Neonatal DM The mutations contributing to TNDM predominantly fall in two major categories- aberrations in 6q24 locus and activating mutations of K_{ATP} channel-related genes (*ABCC8* and *KCNJ11*).

Normally, 6q24 locus is subject to imprinting (by methylation of CpG islands) when maternally inherited. A disorder of imprinting resulting in overexpression of *PLAGL1/ZAC* (pleiomorphic adenoma gene-like 1) and *HYMAI* (Hydatidiform mole-associated and imprinted transcript) genes can cause a functional beta cell defect with variable expression based on age. The mechanisms described are paternal uniparental disomy of

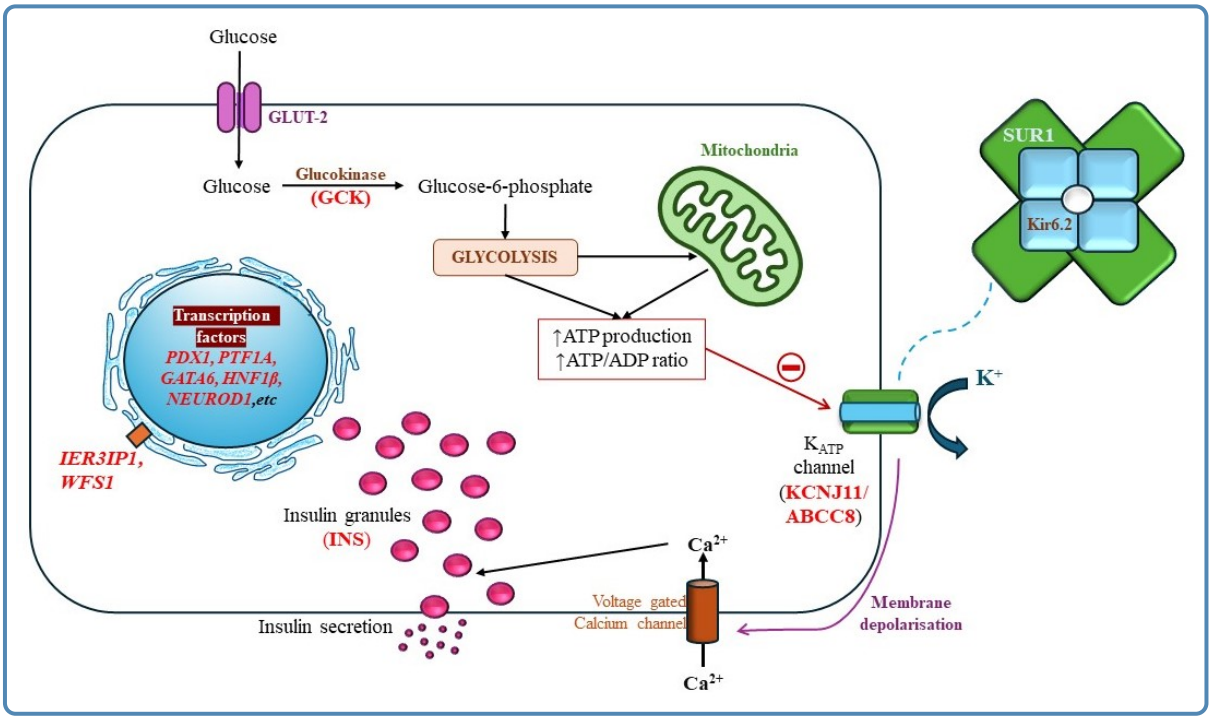

Figure 1 Pictorial representation of glucose response and insulin secretion pathway in the pancreatic beta cells and the genes associated with the pathway.

chromosome 6 (UPD6), paternal duplication of 6q24 and hypomethylation of maternal 6q24.

ZFP57 pathogenic variants contribute to TNDM as part of multi-locus imprinting disturbance with recessive inheritance.

B) Permanent Neonatal DM The mechanism of insulin secretion by beta cells of pancreas is depicted in Figure-1. Any defect in the genes and molecules involved will hamper insulin synthesis or secretion, resulting in neonatal diabetes. These mutations are classified as follows:

1. **Genetic causes of beta cell functional defect**- K_{ATP} channel mutations (*ABCC8/ KCNJ11*), insulin (*INS*) gene mutations with an altered protein expression and glucokinase (*GCK*) mutations

2. **Beta cell destruction by early immune destruction or endoplasmic Reticulum stress**- Accumulation of abnormal proteins because of *INS, EIF2AK3* (enzyme), *IER3IP1* (endoplasmic Reticulum protein) and *WFS1* (Wolframin- ER protein) mutations can cause beta cell apoptosis due to ER stress. Alternatively, immunodysregulation in the IPEX (Immunodysregulation, polyendocrinopathy, and enteropathy, X-linked) syndrome causes immune mediated beta cell destruction.

3. **Endocrine pancreas developmental abnormality**- Transcription factor mutations which are associated with congenital malformations include pathogenic variants of *PDX1, PTF1A, GATA6, GATA4, GLIS3, HNF1β, NEUROD1, NEUROG3,* etc.

Most common mutations are *KCNJ11* (30%), *ABCC8* (19%), *INS* (20%), *GCK* (4%) and *PDX1* (<1%) (De León et al., 2024). Salient clinical features of these pathogenic variants are described in **Table 1**.

K_{ATP} channels consist of the ion transporting Kir6.2 subunits (coded by the *KCNJ11* gene) and regulator subunit SUR1 (sulfonylurea receptor coded by the *ABCC8* gene). Activating heterozygous variants cause membrane hyperpolarisation, and prevent calcium influx and thereby insulin secretion. Since these channels are also present in the brain, neurological symptoms may be seen. Oral sulfonylureas can bind to the SUR1 subunit and induce closure of potassium channels, thereby reestablishing the normal physiology.

Heterozygous variants in the insulin (*INS*) gene affect the structure of preproinsulin. Abnormal protein accumulates, causing severe endoplasmic reticulum stress and β cell death. This variant

Table 1 Salient clinical features of common pathogenic variants of NDM

Gene/ Chromosome	Locus	Clinical features
Chr 6	6q24	• Transient NDM • Onset within 6 weeks • Hyperglycemia, dehydration, and failure to thrive at presentation • Ketoacidosis is rare • Macroglossia or umbilical hernia may be associated. • Remission within 18 months • Recurs in adolescence or adulthood- resembles type 2 diabetes.
ABCC8/ KCNJ11	11p15.1	• Transient/ permanent NDM • Onset before 6 months • IUGR in antenatal period and SGA at birth • Neurological symptoms- attention deficit hyperactivity disorder, sleep disruptions, seizures, and developmental delay
INS	11p15.5	• Transient/ permanent NDM • Presents any time before 1 year • Ketoacidosis at presentation
GCK	7p15.3-p15.1	• Permanent NDM • Presents from even neonatal period • Hyperglycemia of both parents • Heterozygotes present with MODY type 2
PDX1	13q12.1	• Permanent NDM • Agenesis/ hypoplasia of pancreas

NDM- Neonatal diabetes mellitus; IUGR- Intrauterine growth retardation; SGA- Small for gestational age; MODY- Maturity-onset diabetes of the young

usually causes permanent NDM. However, certain rare, recessive mutations are also reported, which alter the protein expression and can cause transient or permanent DM. These respond well to insulin therapy.

The first step in glucose metabolism inside β cell is catalysed by the glucokinase enzyme (coded by the *GCK* gene). It is a "sensor" of blood glucose and controls insulin secretion. Nonsense mutations cause neonatal diabetes when homozygous as glucokinase is completely deficient. Similar heterozygous mutations can

cause glucose intolerance (MODY 2) and hence parents of those with homozygous mutations can have fasting hyperglycemia.

Homozygous mutations of *PDX1* gene can present with pancreatic agenesis or hypoplasia. **Table 2** lists the other significant pathogenic variants.

Clinical features

Insulin being an anabolic hormone plays a critical role in fetal and extrauterine growth. In this context of insulin deficiency, many patients present with intrauterine growth retardation and low birth weight. Postnatal faltering of growth manifests when untreated. Clinical differentiation of transient from permanent NDM is difficult, except for the rapid fall in insulin requirement over 12-14 weeks. However, findings such as macroglossia and umbilical hernia have been described in 6q24-associated phenotypes. Fifty to sixty percent of TNDM cases can present with relapse of diabetes around puberty and in adulthood, which resembles early onset type 2 diabetes mellitus (Temple and Shield, 2002). This is proposed to be due to insulin resistance and could be prevented with lifestyle modifications and avoiding the potential risk factors (fast-food, smoking, lack of exercise). Neurological features like developmental delay and epilepsy (DEND syndrome), attention-deficit hyperactivity disorder (ADHD) or sleep disruptions are suggestive of K_{ATP} channel mutations. Ketoacidosis is rare at presentation and almost unlikely in transient NDM. Around 30% of individuals with *INS* mutations present with diabetic ketoacidosis (DKA) (Letourneau et al., 2017). The specific gene-related clinical features are listed in **Tables 1 and 2**.

Diagnosis of NDM

Hyperglycemia in the neonatal period may be caused by prematurity, extremely low birthweight, sepsis, necrotising enterocolitis, parenteral nutrition, use of drugs (such as glucocorticoids, catecholamine, caffeine, etc.) and any forms of stress such as mechanical ventilation or surgery. Neonatal DM is a rare cause, but there should be a strong suspicion when persistent (>150-200 mg/dL and insulin dependent more than seven days) or acute extreme hyperglycaemia (>1000mg/dL) is noted. Low or undetectable plasma insulin and C-peptide levels relative to hyperglycemia can confirm the diagnosis of NDM. Hyperketonaemia and ketonuria are not usually seen in initial presentation. An abdominal ultrasonogram must be done to look for presence or absence of pancreas. Further delineation of pancreatic morphology is done with computed tomography (CT) or magnetic resonance imaging (MRI) of the pancreas, whenever indicated. Stool fat examination and fecal elastase is tested in those with pancreatic agenesis/ hypoplasia to rule out exocrine deficiency.

Molecular genetic testing is recommended for all diabetes mellitus detected in less than 6 months of age. Additionally, those presenting between 6 months and 1 year should be tested if any extra-pancreatic features, negative autoantibodies, unusual family history, associated congenital defects or multiple autoimmune disorders are noted (ISPAD Clinical Practice Consensus Guidelines, 2022). Different testing strategies are used (**Figure 2**), such as serial single gene testing, multigene panel including the most common genes like *ABCC8, KCNJ11, INS, GCK* and *PDX1*, or a comprehensive genomic testing with whole exome or whole genome sequencing. Syndrome specific clinical phenotypes should be tested for the corresponding gene as per **Table 2**. Serial testing of single genes is a time consuming and expensive procedure with a high chance to miss rare genetic variants, except when clinical phenotypes specific to certain genes are identified. Whole exome sequencing is a cost-effective approach in a country like India, enabling detection of both common and uncommon pathogenic variants.

Management of NDM

Initial management consists of emergency stabilisation by rehydration and intravenous insulin infusion to control hyperglycemia. Once the child is stable and tolerates oral feeds, an appropriate regimen of subcutaneous insulin must be started. Infants requires very minimal doses of insulin only and hypoglycemia is more dangerous to the growing brain. Any inappropriate dose of rapid and short acting insulin can cause severe hypoglycemia and should be avoided. Longer acting insulin analogues such as Glargine or Detemir are preferred, as it maintains a basal insulin level without significant hypoglycemia. Intermediate acting insulins are not as effective but may be used in low-income settings.

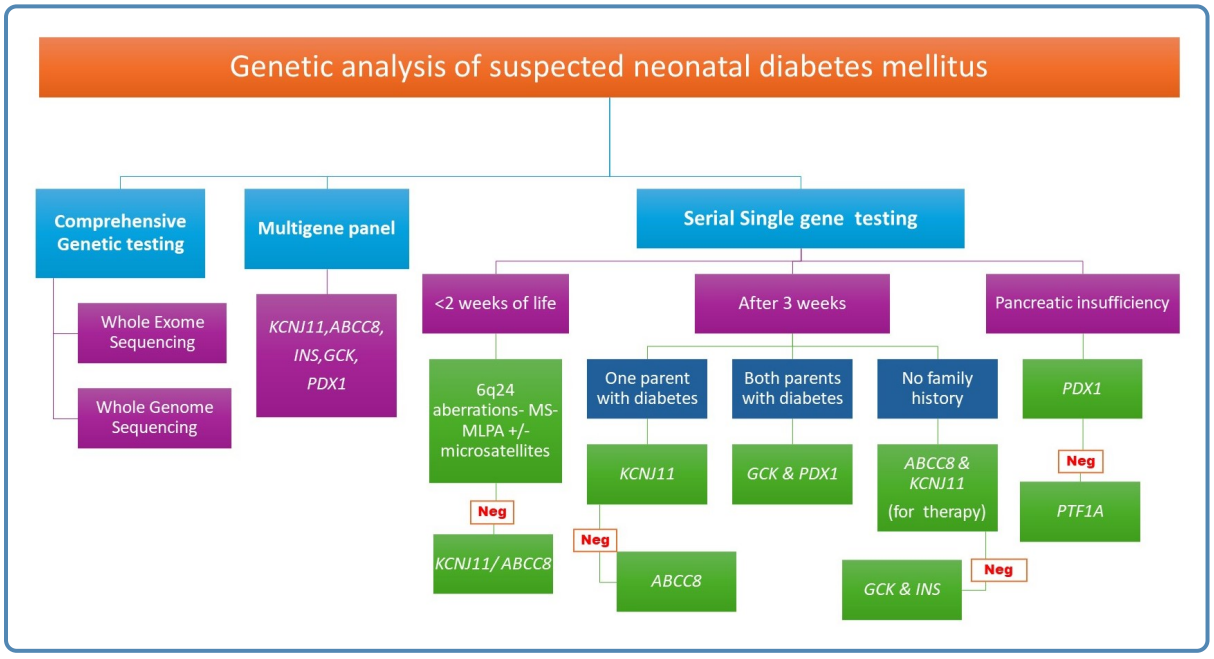

Figure 2 Algorithm for genetic testing of suspected neontal diabetes

Continuous subcutaneous insulin infusion (CSII) can deliver accurate insulin doses corresponding to blood glucose levels. This is more physiologic, safer, and reduces HbA1c better when compared to other regimens.

Sulfonylurea therapy is beneficial in *KCNJ11* and *ABCC8* mutations and some pathogenic variants of *GCK*. Around 90-95% of these patients achieve glycemic control with oral sulfonylureas when weaned off insulin therapy. It also improves the neurological symptoms. Various transfer protocols are available online for insulin to sulfonylurea transition. High doses (0.4-1.0 mg/kg/day of Glibenclamide) may be required. Crushed tablets are poorly soluble in water. Recently, a sulfonylurea suspension (Amglidia^{R}) has been approved by the European Medicines Agency (EMA). Common side effects are transitory diarrhoea and nausea.

Diet modification is better than diet restriction in children. A high calorie diet is recommended to maintain adequate weight gain. Pancreatic enzyme replacement must be provided for those with exocrine insufficiency.

Relapse of transient neonatal DM can respond to diet alone or needs addition of oral hypoglycemic agents with occasional insulin requirement.

Genetic Counselling

Confirmation of pathogenic variants can help in several aspects such as provision of targeted gene specific therapeutic modalities, early prediction of other associated system involvement, and for counselling and testing other family members. In transient NDM due to 6q24 mutations, paternal duplications are autosomal dominant, and hence carry a 50% transmission risk if inherited from the father (Temple and Shield, 2002). UPD6 is usually sporadic with a low recurrence risk. Maternal hypomethylation can be theoretically transmitted to 50% offspring of affected female individuals, but only de-novo and non-recurrent cases have been detected till now. Pathogenic variants of *KCNJ11* have autosomal dominant inheritance when familial, but 90% are de novo heterozygous mutations (ISPAD CPCG 2022). Phenotypes related to *ABCC8* and *INS* variants are either autosomal dominant or recessive and *GCK* and *PDX1*- related NDM follows an autosomal recessive pattern of inheritance.

Genetic counselling begins with identification of the variant(s) in the proband. There are several methods available to determine the pathogenicity of the variant such as database searches, in silico modelling with web-based applications, in-vitro functional studies of the protein product and

Table 2 Molecular genetics of syndromic forms of neonatal diabetes

Clinical phenotype	Genes affected	OMIM Phenotype
Pancreatic exocrine insufficiency or agenesis and cardiac abnormalities	*GATA6*	Neonatal and childhood Onset diabetes/ Pancreatic agenesis and congenital heart defects (OMIM # 600001)
Enteropathy and dermatitis	*FOXP3*	Immunodysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX syndrome) (OMIM # 304790)
Cerebellar involvement	*PTF1A*	Pancreatic agenesis 2/ Pancreatic and cerebellar agenesis (OMIM # 609069)
Congenital hypothyroidism, hepatic fibrosis, cystic renal dysplasia, congenital glaucoma	*GLIS3*	Diabetes mellitus, neonatal, with congenital hypothyroidism (OMIM # 610199)
Cerebellar hypoplasia, sensorineural deafness, and visual impairment	*NEUROD1*	Maturity-Onset Diabetes of the Young 6 (MODY 6) (OMIM # 606394)
Pancreatic hypoplasia, intestinal atresia, and gall bladder hypoplasia	*RFX6*	Mitchell-Riley syndrome (OMIM # 615710)
Congenital malabsorptive diarrhoea	*NEUROG3*	Diarrhea 4, malabsorptive, congenital (OMIM # 610370)
Skeletal abnormalities (epiphyseal dysplasia) and liver dysfunction	*EIF2AK3*	Wolcott-Rallison syndrome (OMIM # 226980)
Megaloblastic anemia and deafness	*SLC19A2*	Thiamine-responsive megaloblastic anemia (TRMA) syndrome (OMIM # 249270)
Renal and genital abnormalities	*HNF1B*	Renal cysts and diabetes syndrome (OMIM # 137920)
Optic atrophy, diabetes insipidus and deafness	*WFS1*	Wolfram syndrome 1 (OMIM # 222300)

clinical studies such as familial co-segregation studies. The mode of inheritance is communicated, and recurrence risk of parents and siblings are predicted. Pathogenic variants of *GCK, INS, PDX1, RFX6*, etc. can present with neonatal diabetes when homozygous and milder forms of diabetes such as Maturity Onset Diabetes of Young (MODY) when heterozygous. In these cases, heterozygous parents and siblings should undergo a screening blood glucose test even if asymptomatic. Prenatal counselling is advised for those with a known pathogenic variant in a family member. Option of prenatal/ preimplantation genetic testing can be suggested for the identified variant in the proband.

Follow–up and surveillance

Periodic daily blood glucose monitoring with conventional glucometers or continuous glucose monitoring systems (CGMS) is done to assess therapeutic adequacy. Target HbA1c should be

less than 7.5. ISPAD suggests yearly HbA1c monitoring for transient NDM patients after remission for early identification of relapse. Long term follow-up should include metabolic work-up and socio-education. Periodic developmental assessment is needed, especially in *KCNJ11* and *ABCC8* pathogenic variants. Yearly screening for microalbuminuria and retinopathy should start from 10 years of age.

Conclusion

Neonatal diabetes mellitus is a rare cause of hyperglycemia in neonates, with a predominant monogenic origin. After confirming the diagnosis, genetic testing is recommended for prognostication and management. Pancreatic malformations and extra-pancreatic features should be specifically looked for. Initial therapy is with insulin replacement, but a transition to oral sulfonylureas must be attempted as soon as a favourable pathogenic variant (*KCNJ11* and *ABCC8*) is identified. Lifestyle modifications and periodic follow-up are the key to disease control.

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GeNeXpreSS

Novel Tools for Detecting Structural Variants: Optical Genome Mapping & More

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The impact of inversions across 33,924 families with rare disease from a national genome sequencing project (Pagnamenta et al., 2024)

Genomic inversions, segments of DNA with reversed orientation compared to the reference genome, are prevalent in human populations and vary in size. Traditional karyotyping can detect these inversions but typically misses those smaller than 10 Mb. Despite advancements in array technologies, copy-neutral structural variants (SVs), such as inversions, remain underexplored in clinical settings. This study aimed to expand the analysis to 33,924 families in the 100,000 Genomes Project (100kGP), examining genes associated with haploinsufficiency (HI). Structural variants were identified using Canvas and Manta, with prioritization of ultra-rare inversions. Ultimately, only 45 families were identified, representing 1-2% of diagnoses across 351 genes. Notable detected inversions included an intragenic *MSH2* founder inversion, a complex maternally inherited structural variant, a de novo inversion in the *HOXD* cluster linked to Kantaputra-type mesomelic dysplasia, and an inversion with a breakpoint in intron 4 of the *APC* gene indicating potential gene disruption. Limitations included a focus on HI genes and potential oversight of inversions in repetitive regions.

A comparison of structural variant calling from short-read and nanopore-based whole-genome sequencing using optical genome mapping as a benchmark (Pei et al., 2024)

This study aimed to assess the clinical efficacy of three genomic technologies: Illumina short-read whole genome sequencing (SR-WGS), Oxford Nanopore Technologies long-read whole genome sequencing (LR-WGS), and Bionano optical genome mapping (OGM) for detecting rare structural variants (SVs) of potential clinical significance. The investigation centered on a model cohort of patients affected by craniosynostosis (CRS), a condition known for its considerable clinical and genetic heterogeneity. As part of the 100,000 Genomes Project (100kGP), 114 CRS families that lacked a genetic diagnosis were recruited and sequenced using Illumina SR-WGS technology. In spite of thorough investigations focused on uncovering causative single nucleotide polymorphisms (SNPs) and structural variants (SVs), 78 families continued to lack a diagnosis. From these families, nine trios were selected. By integrating analyses from LR ONT WGS and Bionano OGM, the uncovered SVs that may have been overlooked by Illumina WGS were considered. A subset of potentially clinically relevant rare SVs was identified through Bionano OGM, which was utilized to create a "truth dataset" for benchmarking the performance of current variant callers from Illumina and ONT to evaluate their clinical utility in rare disease contexts.

VolcanoSV enables accurate and robust structural variant calling in diploid genomes from single-molecule long-read sequencing (Luo et al.,2024)

Advances in long-read sequencing technologies have provided a valuable resource for comprehensive SV detection. However, accurately identifying SV breakpoints and sequences remains challenging. To address this, researchers have developed innovative hybrid SV detection pipelines that utilize both reference genomes and local de novo assembly. One such tool, VolcanoSV, employs phased single nucleotide

polymorphisms (SNPs) and unique k-mer similarity analysis to enable precise haplotype-resolved SV discovery. VolcanoSV constructs comprehensive genetic maps encompassing SNPs, small indels, and all types of SVs, making it well-suited for human genomics studies. Extensive experiments have demonstrated that VolcanoSV surpasses state-of-the-art assembly-based tools in detecting insertion and deletion SVs, exhibiting superior recall, precision, F1 scores, and genotype accuracy across diverse datasets, including low-coverage (10x) datasets. Additionally, VolcanoSV outperforms other tools in identifying complex SVs, such as translocations, duplications, and inversions, in both simulated and real cancer data. The pipeline is also robust to various evaluation parameters and accurately identifies breakpoints and SV sequences.

Optical genome mapping unveils hidden structural variants in neurodevelopmental disorders (Schrauwen et al., 2024)

This study explored the application of optical genome mapping (OGM) in identifying structural variants (SVs) associated with neurodevelopmental disorders (NDDs) that remain undetected by standard exome sequencing. OGM, which surpasses short-read sequencing in capturing complex SVs, was conducted on ultra-high molecular weight DNA from 47 families. OGM analysis of the 47 unsolved families revealed that the majority of identified variants consisted of insertions (67.6%) and deletions (29.2%). Among these families, OGM identified 7 rare variants of interest, including 2 variants of unknown significance and 5 likely pathogenic or pathogenic structural variants (SVs). These likely pathogenic or pathogenic SVs were found in known neurodevelopmental disorder (NDD) genes, such as *BCL11A*, *OPHN1*, *PHF8*, *SON*, and *NFIA*. Additionally, an inversion affecting the *NAALADL2* gene was identified, previously linked to complex rearrangements in NDD cases. The variants missed by exome sequencing primarily included larger insertions (>1 kbp), inversions, and small deletions/duplications (1-4 exons). OGM not only enhances molecular diagnostics for NDDs but also has the potential to uncover novel NDD-related genes harbouring complex SVs often overlooked by conventional sequencing methods.

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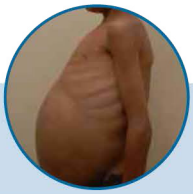

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