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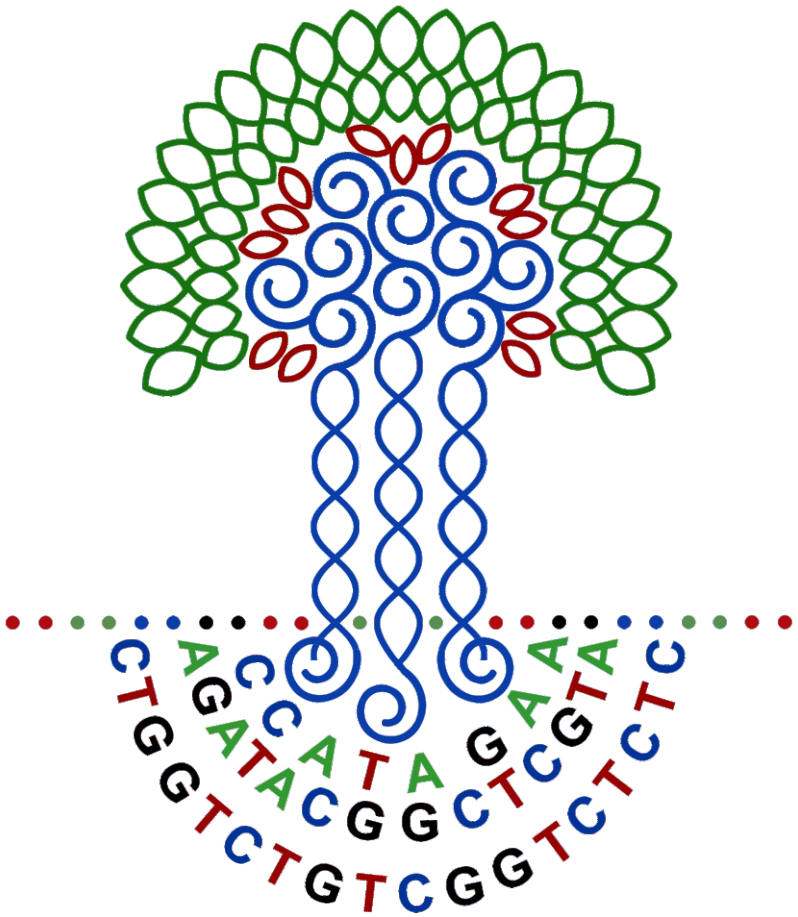
Table of Contents



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PhotoQuiz - 61

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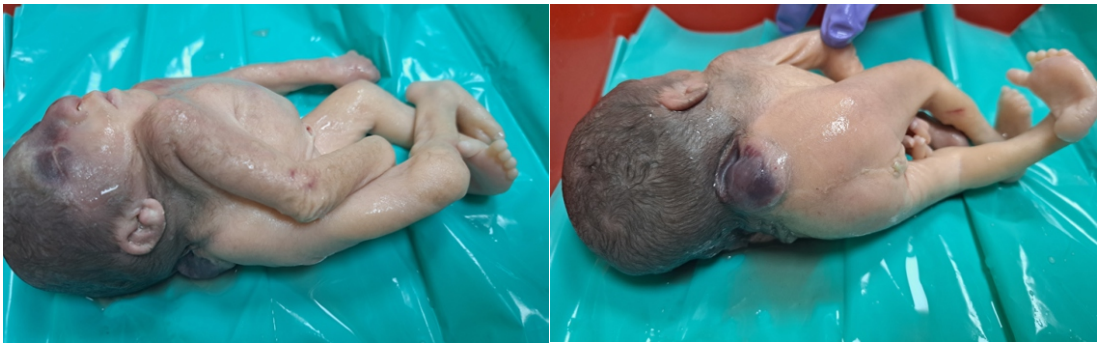
This fetus with antenatally detected anomalies was referred for autopsy evaluation. Identify the condition.

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

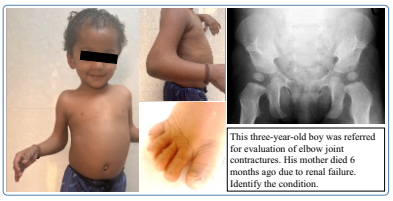


Answer to PhotoQuiz 60

Nail-patella syndrome (OMIM #161200)

Nail-patella syndrome is an autosomal dominant disorder characterized by nail abnormalities, small or absent patellae, elbow joint contractures, and the presence of iliac horns. Nails may be absent, hypoplastic, dystrophic, ridged, pitted, or discolored. Iliac horns are seen as projections arising from the central part of the iliac bones of the pelvis. Affected individuals can have renal involvement and develop proteinuria, hematuria, and end-stage renal disease. Nail-patella syndrome is caused by heterozygous pathogenic variants in the *LMX1B* gene (*602575).

Correct responses were given by:



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GeNeDit

The Long and Short of NGS

Editorial

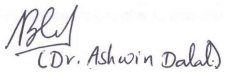
Next-generation sequencing (NGS) has revolutionized the field of genetic diagnostics over the last 10-15 years. NGS technologies have enabled us to look at the DNA at base pair level of resolution at genome wide scale as opposed to the very low-resolution techniques like karyotyping wherein the smallest abnormality which could be detected was around 5 million bases. Sanger sequencing technology enabled us to look at the DNA at base pair level for small sequences of up to 1000 bases. Repetitive Sanger sequencing for large number of fragments was the strategy used to sequence the first human genome in 2003 with a cost of about 3 billion dollars. Recently available NGS technologies have enabled sequencing of the whole human genome for as low as $100 due to a drastic drop in the cost of sequencing. Most of the contemporary NGS technologies are based on 'short-read sequencing' wherein the DNA is fragmented into small pieces of about 250-300 bases and all such fragments are sequenced in a massively parallel sequencing. These short reads are then aligned to the reference genome using various bioinformatic tools and variants are identified by comparison of sequences with those in the reference genome. Short-read sequencing has been used in research as well as in the clinic for sequencing of groups of genes (panel sequencing), exome sequencing or genome sequencing. Short-read sequencing methods can detect single nucleotide variations and small insertions/deletions with a high degree of accuracy and hence have helped in the diagnosis of a large number of genetic disorders with a diagnostic yield of about 30-70% depending on the indication. Although short-read sequencing technologies have been successfully used for genetic diagnostics over the last few years, there are certain drawbacks of this technology. Structural variations like large deletions/duplications, translocations, insertions, triplet repeat expansions/contractions etc. are not detectable by short-read sequencing with sufficient accuracy.

The disadvantages associated with short-read sequencing have led to the development of 'long-read sequencing' methodologies wherein large fragments of DNA ranging from few kilobases to megabases can be sequenced as a single strand. This enables the ability to detect structural variations and triplet repeat expansions with greater accuracy compared to short-read sequencing. The two majorly used long-read sequencing technologies are Oxford Nanopore and Pacific Biosciences based techniques. However, the long-read sequencing technologies suffer from lower accuracy for single nucleotide variations and small insertions/deletions. The ultimate ideal tool for DNA sequencing would be the one which can provide highly accurate long-read sequences so that all types of genetic variations can be detected in a single test and that too at a low cost. Newer advances in long-read sequencing technologies are enabling the increase in accuracy for small variants through use of better techniques as well as use of consensus sequences derived from iterative sequencing. The GenExpress in this issue discusses a few articles wherein long-read sequencing technologies have enabled detection of variants in genetic conditions like Duchenne muscular dystrophy (DMD), triplet repeat expansion disorders, etc. resulting from large rearrangements not detectable using short-read sequencing. Of interest is the article published by Dutta et al wherein long-read sequencing helped in detection of the breakpoints, at base-level resolution, of a de-novo reciprocal translocation.

The field of genetics is ever evolving and long read sequences can only allow us to look at DNA fragments of 1-2 Mb length, at the maximum capacity. However, the length of the human chromosomes ranges from few 50 - 250 Mb. The best technology available to look at large chromosomal rearrangements is karyotyping but its resolution is only around 5 Mb. Hence, there was a need for a technology to look at chromosomes at high resolution in order to detect large structural rearrangements and the same

was fulfilled by a recently developed technology called optical genome mapping (OGM). OGM involves labelling of DNA using fluorescent markers and then visualization of large fragments of DNA of hundreds of megabases. OGM is the only technique other than the cumbersome and low-resolution Southern blot technique, which can detect contraction of repeats in facioscapulohumeral muscular dystrophy (FSHD). These aspects of OGM technology have been highlighted in the article by Tallapaka et al in this issue.

It is an exciting time for medical genetics as newer technologies are enabling us to look at the human DNA in much more detail which is likely to help in the identification of genetic variants in all patients with genetic disorders as well as paving the way for high quality research.



Dr Ashwin Dalal
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1st July 2023

Book Review

Book Review

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Review on: Diagnosis and Management of Hereditary Cancer - Tabular-Based Clinical and Genetic Aspects. By John W Henson, MD and Robert G Resta, MS. Academic Press, Elsevier, 2021.

With the discovery of BRCA genes, interest in hereditary cancer has mounted in all countries. However, most physicians and oncologists do not have enough knowledge of genetic concepts and their clinical applications. The physicians are also always short of time. This book produced in tabular form is most suitable for busy clinicians. The initial chapters provide information regarding concepts in hereditary cancer, genetic alteration in cancer, genetic counseling, and genetic testing. Detection of variants, and their interpretation as per the guidelines of the American College of Medical Genetics and Genomics (ACMG) is outlined. Topics such as oncogenes, autosomal recessive (AR) hereditary cancer syndromes, non-neoplastic conditions associated with AR cancer genes e.g., *ATM*, fumarate deficiency, Nijmegen breakage syndrome etc., and digenic modifiers of *NF1*, *TSC*, retinoblastoma, and *VHL* are covered. Factors which affect phenotypic variability such as molecular complexity, digenic effect, epimutations and environmental influences, haplo-insufficiency, mosaicism etc. are tabulated.

The section on red flags is very good and should be compulsory reading for all physicians. Issues that may affect evaluation of family history are covered. Pedigree symbols and definitions are enumerated. Features observed on physical examination and the disorders with which they are associated are listed in a useful table. Risk estimation models are explained briefly, although these are mostly based on European ancestry.

Screening, surveillance, and diagnostic tests for cancer [based on blood, urine, magnetic resonance imaging (MRI), ultrasound endoscopy, positron emission tomography (PET) scan etc.] are presented. Physiologic imaging tests for phaeochromocytoma, paraganglioma and neuroendocrine tumors are defined. Management of known alterations through chemoprevention, chemotherapy, colectomy, mastectomy, hysterectomy, gastrectomy, risk reduction oophorectomy etc. are detailed.

Section D covers tumor syndromes-related genes in some detail. Section E describes classic hereditary cancer syndromes starting from APC-associated polyposis, hereditary breast and ovarian cancer (HBOC) to renal cell carcinoma. Some patient care plans are presented, followed by information sources. Overall, the book fulfills the need of providing essential information on hereditary cancer for the busy clinician.

Clinical Vignette

GJC2 Variant Identification in Siblings with Pelizaeus–Merzbacher-Like Disease: Illustrative Report Highlighting the Limitations of Exome Sequencing

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Abstract

Whole-exome sequencing (WES) has revolutionized genetic diagnosis and has become a powerful tool for identifying disease-causing variants. However, WES has limitations such as uneven read coverage, which can result in multiple low-coverage regions in the exome. Here, we report two affected siblings with a phenotype consistent with Pelizaeus-Merzbacher-like disease 1 (PMLD1), a rare early-onset autosomal recessive disorder caused by biallelic variants in the *GJC2* gene. WES analysis of one affected child initially revealed only a single heterozygous nonsense variant in exon 2 of the *GJC2* gene. However, on reanalysis with a local de novo assembly approach using the GATK HaplotypeCaller, a second variant - a frameshift insertion - was subsequently identified. This variant was found to be present in a low coverage region which is why it was missed in the initial analysis. Analysis of the segregation pattern of the variants in the affected sibling and parents through targeted Sanger sequencing confirmed that the variants were present in compound heterozygous form in both the affected siblings. Our study emphasizes the importance of considering the limitations of exome sequencing, especially in terms of low coverage of certain exonic regions.

Keywords: Pelizaeus-Merzbacher-like disease 1, *GJC2* gene, whole-exome sequencing, gnomAD, GATK HaplotypeCaller

Introduction

Pelizaeus-Merzbacher-like disease-1 (PMLD1), also referred to as hypomyelinating leukodystrophy type 2 (HLD2), is a rare autosomal recessive neurological disorder caused by abnormal formation or maintenance of the myelin sheath that surrounds and protects nerve fibers. This can lead to impaired nerve signal transmission and manifestation of disease symptoms such as developmental delay, progressive spasticity, motor impairment, ataxia and nystagmus, and findings of hypomyelination on magnetic resonance imaging (MRI) of the brain (Nahhas et al., 2003). Here, we report a family with Pelizaeus-Merzbacher-like disease 1 (PMLD1), where initial analysis of whole-exome sequence data identified only one heterozygous pathogenic variant, but subsequent reanalysis detected the second variant in a low-coverage region of the gene.

Patients and Methods

The proband (P1), an 8-year-old male child, was the fourth offspring of non-consanguineous parents (**Figure 1**). He was born at term gestation with a birth weight of 2.5 kg and had no history of prenatal or postnatal complications. During infancy, he had hypotonia and motor developmental delay, with delayed neck holding attained at 11 months and delayed walking without support attained at 2 years. He also had poor vision since infancy. He had regression of his motor milestones, starting from the age of around 7.5 years. There was no history of seizures or abnormal movements. The language milestones

and intellectual development were normal (IQ-73 at the age of 7 year 7 months). His height was 132 cm (Z score of 0.66), weight was 31 kgs (Z score of 0.87) and head circumference was 49.5 cm (Z score of -2). On examination, he had reduced vision and nystagmus was present. Increased muscle tone was noted in all four limbs with exaggerated deep tendon reflexes, positive ankle clonus and bilateral extensor plantar reflex. Other organ systems were normal on clinical examination. Ophthalmology evaluation revealed optic atrophy with nystagmus. MRI of the brain showed homogenous T2 hyperintensities involving the central and peripheral aspects of the cerebral white matter as well as the pons and medulla regions, mild vermian atrophy, and hypoplastic corpus callosum. Based on the clinical and neuroimaging features, the diagnosis of hypomyelinating leukodystrophy was suspected in the proband.

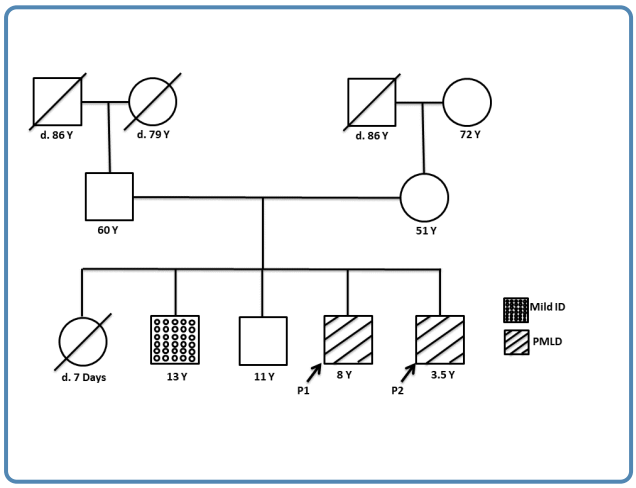

Figure 1 The pedigree of the family affected with Pelizaeus- Merzbacher -like disease showing the affected individuals, the unaffected sibling, and the parents.

His similarly affected 3.5-year-old male sibling (P2) presented with motor developmental delay since infancy along with nystagmus; his speech was age-appropriate.

WES analysis of the proband P1 initially revealed only a single heterozygous nonsense variant (GJC2:c.852T>A:p.Cys284Ter) in exon 2 of the *GJC2* gene. Subsequent reanalysis of the WES data revealed the second variant which was a novel frameshift insertion variant (GJC2:c.907_923dup:p.Pro309fsTer168) also in exon 2 of the *GJC2* gene. According to the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines, both variants are classified as 'likely pathogenic' as they meet the PVS1 criterion for null variants in a gene where loss-of-function is a known mechanism of disease, and the c.852T>A variant also meets the PM2 criterion for being absent in large population databases (Richards et al., 2015). Notably, the frameshift variant had low coverage (depth: 10X) and was not found in the bam file generated by GATK BaseRecalibrator but was identified in the HaplotypeCaller bam, as depicted in **Figure 2**.

The compound heterozygous status of the affected siblings and the heterozygous carrier status of the unaffected sibling and both parents were confirmed by targeted Sanger sequencing, supporting an autosomal recessive mode of inheritance (**Figure 3**).

Discussion

The *GJC2* gene, which is highly expressed in oligodendrocytes, encodes for the connexin-47 (Cx47) protein that forms gap junction channels allowing intercellular communication between oligodendrocytes and astrocytes by heterotypic coupling (Cx47-Cx43 channels) (Kleopa et al.,2004; Qju et al., 2022). Variants in *GJC2* can result in loss of function of the transmembrane protein, which may be attributed to altered channel properties or impaired protein trafficking to the cell membrane (Biancheri et al.,2013; Owczarek-Lipska et al., 2019). Exon 2 of *GJC2*, which codes for majority of the Cx47 protein, contains two low-coverage regions in WES data due to its GC-rich sequence. To identify such variants, it is important to examine the bam file generated by GATK HaplotypeCaller, which uses a local de novo assembly approach to detect variants, including small insertions and deletions (indels). Nonetheless, the detection sensitivity of the HaplotypeCaller may vary depending on factors such as sequencing data quality and depth of coverage.

A general low coverage of exon 2 region of *GJC2* is present in the gnomAD v2.1.1 exome sequencing dataset (https://gnomad.broadinstitute.org), which may be due to the high GC content of this region (Karczewski et al., 2020). However, the gnomAD genome sequencing dataset shows uniform coverage in the same region, suggesting

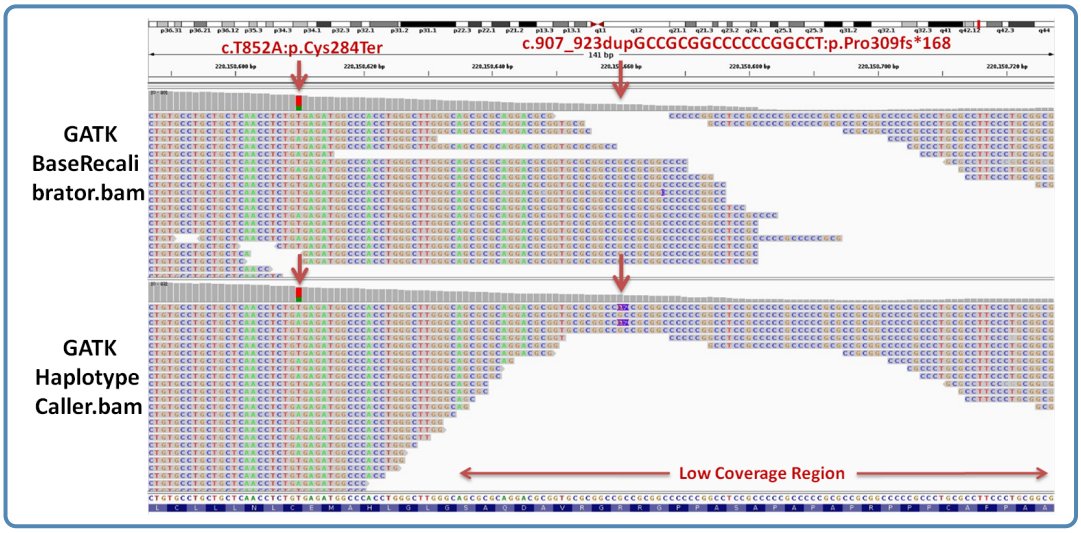

Figure 2 Visualization of bam files generated from the GATK BaseRecalibrator and HaplotypeCaller, using Integrative Genomics Viewer (IGV).

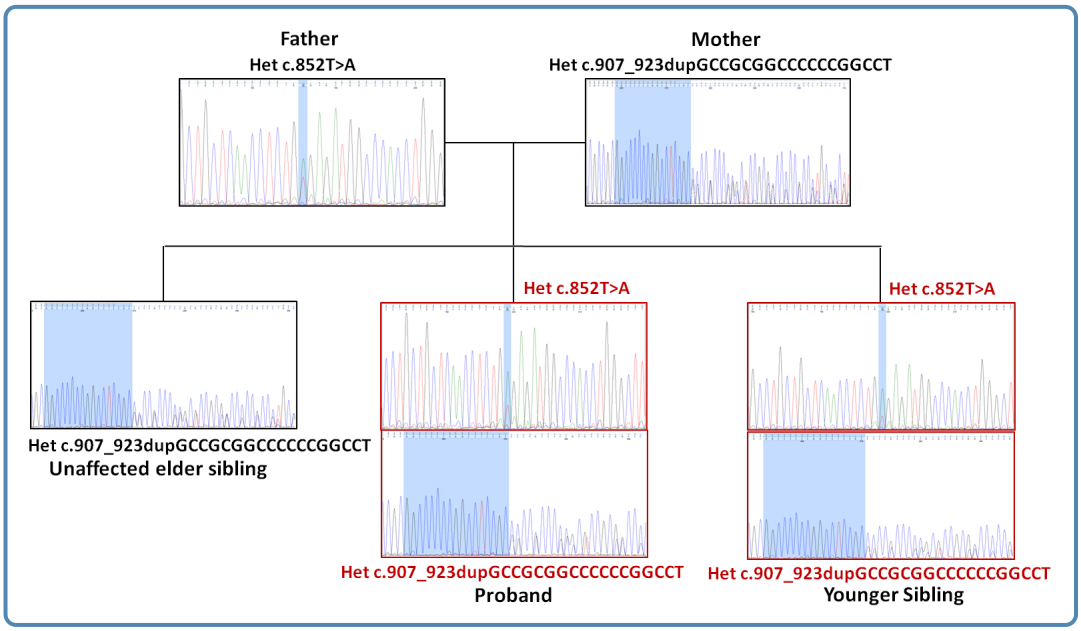

Figure 3 Segregation analysis of the *GJC2* compound heterozygous variants within the family, as confirmed by targeted Sanger sequencing.

that the low coverage in the exome dataset may be due to limitations of exome sequencing (**Figure 4**). Variants within such low-coverage regions can be difficult to call, which makes the uniformly covered exome 'extracted' from the genome sequencing data a helpful approach in such cases. Exome sequence data 'fetched' from genome sequencing data facilitates the identification of such variants that may otherwise be missed in the exome regions.

The Cx47 protein is composed of two extracellular, four transmembrane and three cytoplasmic domains. The compound heterozygous variants identified in this family affect the third cytoplasmic domain of Cx47. Specifically, the nonsense variant (p.Cys284Ter)

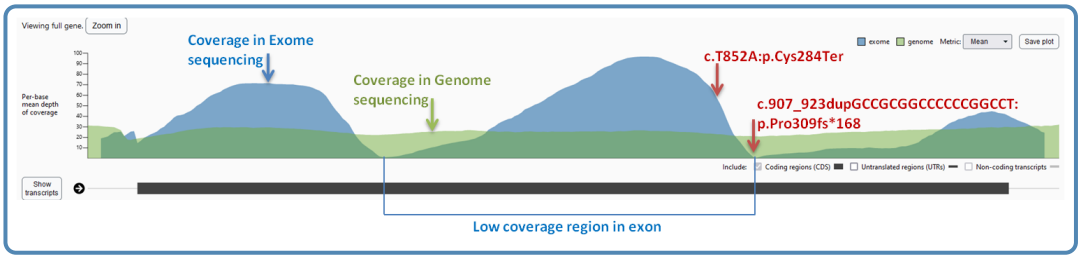

Figure 4 The coverage plot of GJC2 gene in gnomAD v2.1.1. The X-axis represents the genomic position along the exon2 of GJC2, while the Y-axis represents the depth of coverage. The blue and green regions represent the coverage obtained from exome and genome sequencing datasets. The variant positions are indicated by arrows.

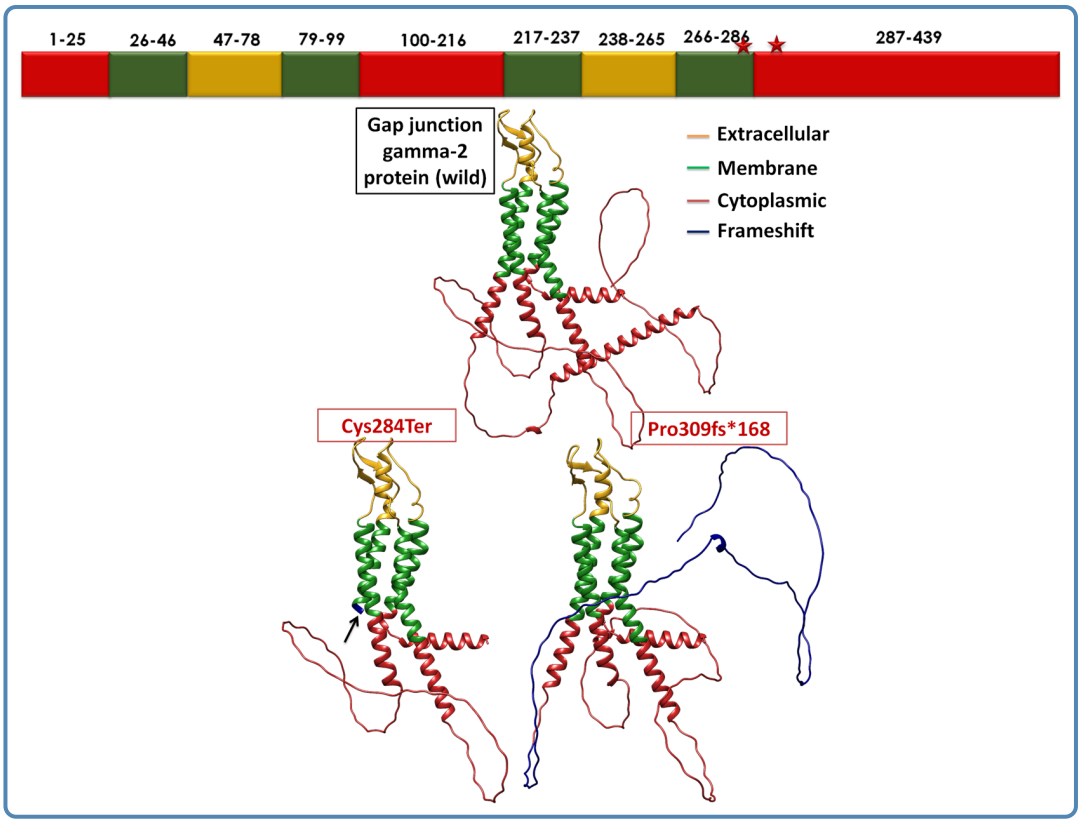

Figure 5 GJC2 protein representation in three forms: wild-type, and in variants p.Cys284Ter and p.Pro309fsTer168. The extracellular domains are highlighted in mustard yellow, transmembrane domains in green, and the cytoplasmic domain in red. Both nonsense and frameshift mutations are represented in blue.

truncates the protein just before the third cytoplasmic domain, while the frameshift insertion (p.Pro309fs) is predicted to alter subsequent amino acids and produce an elongated tail of 38 additional residues in the third cytoplasmic domain (**Figure 5**). Hence, these variants can have a significant impact on the structure and function of the Cx47 protein.

Conclusion

This report highlights the limitations of exome sequencing in detecting genetic variants in certain

exonic regions such as those with a high GC content and those with repeat elements or segmental duplications. Examining the bam file generated by GATK HaplotypeCaller would be helpful for identifying variants in such regions. Exome sequence data extracted from WGS data rather than through WES would be better for such regions due to the uniformity of coverage.

Acknowledgement

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GeNeViSTA

Therapeutic Modalities for Hereditary Angioedema: An Update

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Abstract

Hereditary angioedema is an autosomal dominant disorder presenting as intermittent skin swelling, abdominal pain attacks, and potentially life-threatening upper airway obstruction. Most of the cases are caused by pathogenic variants in the *SERPING1* gene. Other genes contribute to a small number of cases. Mutations in the *SERPING1* gene result in deficiency or altered function of plasma C1 esterase inhibitor (C1 INH), a serine protease inhibitor that normally inhibits proteases in the contact, complement, and fibrinolytic systems. The increased vascular permeability leading to edema is mediated through increased levels of bradykinin. The unravelling of the pathophysiology and mechanism involved in swelling associated with hereditary angioedema (HAE) resulted in the development of therapeutic options for the acute attacks and prophylactic treatment of patients with HAE. Here we present a brief review of the past treatments, current treatment options and potential future treatment modalities for HAE.

Keywords: Hereditary angioedema, C1 esterase inhibitor, *SERPING1*, Gene therapy

Introduction

Hereditary angioedema (HAE) is a rare, autosomal dominant disorder related to C1 inhibitor (C1-INH). It is characterized by recurrent attacks of localized swelling of different body parts mostly involving the face and limbs. The prevalence of hereditary angioedema is 1 in 50,000 (Santacroce et al., 2023). A large case series of patients with HAE diagnosed based on clinical and laboratory criteria as well as the mutation spectrum of *SERPING1* gene have been reported from India (Jindal et al., 2021; Perumalla et al., 2021). Being rare, HAE is mostly misdiagnosed due to symptoms mimicking other disorders involving edema of body parts. There is a mean delay of more than 10 years between the onset of symptoms and diagnosis due to lack of awareness and diagnostic facilities. Most deaths occur in undiagnosed cases since conventional therapies such as corticosteroids and antihistaminics are ineffective. Involvement of larynx or tongue may be life-threatening. There are three types of HAE depending on the etiology. Therapies have become available for the treatment of episodes and short or long-term prophylaxis. Awareness of this rare disease is important for correct and timely diagnosis and initiation of lifesaving treatments.

Etiolopathology of HAE

Based on quantitative or qualitative defects of the C1-inhibitor, HAE has been categorized as HAE with deficient C1-INH (type I) (MIM #106100), HAE with dysfunctional C1-INH (type II), and HAE with normal C1-INH. More than 150 different pathogenic variants have been identified in patients with type I and II HAE. Type I HAE results from a quantitative deficiency of C1-INH due to variants in the *SERPING1* gene, accounting for approximately 85% of cases. In type I HAE, the different *SERPING1* mutations result in truncated or misfolded proteins that cannot be secreted; hence a deficiency in C1-INH level. Type II HAE which has dysfunctional C1-INH protein is responsible for approximately 15% of cases. In type II, *SERPING1* mutations include residues at or near the active site on the reactive mobile loop that result in a mutant C1-INH protein which is secreted but dysfunctional. As a result, type II HAE has normal or even elevated C1-INH with decreased protein function (Zhang et al., 2018). Variants in six other different genes have been identified in affected individuals with normal C1-INH (HAE III). These genes are in the factor XII (*F12*), plasminogen (*PLG*), angiopoietin 1 (*ANGPT1*),

kininogen 1 (*KNG1*), myoferlin (*MYOF*), and heparan sulfate (HS)-glucosamine 3-O-sulfotransferase 6 (*HS3ST6*) and mostly act through plasmin.

Clinical manifestations of HAE

The onset of symptoms of HAE is around adolescence or during childhood in most cases. It manifests with any combination of painless, non-pruritic, nonpitting swelling of submucosal, dermal, or cutaneous tissue with or without severe abdominal pain, or acute airway obstruction due to laryngeal edema. The attacks last for 2 to 5 days, usually slowly increasing and then resolving even without therapy. Triggers for acute attacks of HAE are trauma, infections, stress, or procedures in 40% of cases. Other triggers include ACE inhibitors and estrogens. Prodromal symptoms such as rash (erythema marginatum) occur before majority of attacks. The most frequent sites of swelling include the skin (100%), the abdomen (97%), and the larynx (54%). The life-threatening episodes of laryngeal edema are not infrequent. The disease-associated mortality rates can be up to 33% in hospitalized patients (Agostoni et al., 1992).

Diagnosis of HAE

The inappropriately increased levels of bradykinin along with low levels of C4, C1-INH protein and/or C1-INH function is diagnostic. Low serum C1–INH level is characteristic of HAE I. In type II HAE the quantitative level of C1-INH is normal but functional assay shows deficiency of C1-INH function. The diagnosis is based on laboratory assessment as shown in **Table 1**. C1-INH deficiency or dysfunction increases bradykinin production by inhibiting proteases involved in the complement, contact-system, coagulation, and fibrinolytic pathway (**Figure 1**). The critical functional threshold for C1-INH control of the plasma contact system is approximately 40%.

The acquired angioedema (AAE) with C1 esterase inhibitor deficiency associated with underlying malignancy or rheumatologic disease, angiotensin-converting enzyme inhibitor-induced angioedema, and hypersensitivity reactions or urticaria/angioedema syndromes needs to be considered as differential diagnosis in appropriate clinical scenarios. The acquired type has histamine as the main mediator while in HAE, increased bradykinin is responsible for recurrent episodes of swelling.

Management of HAE

The unavailability of specific therapy leads to significant morbidity and mortality even in diagnosed cases. In most cases, there is inappropriate and rampant use of antihistaminics, corticosteroids, adrenaline, etc. Sometimes, misdiagnosis leads to unnecessary surgical intervention in cases with HAE presenting with pain abdomen mimicking acute abdomen. Often the recurrent nature of angioedema attacks with no proper treatment modalities leads to self-searching for unconventional treatment options by the patients. The utility of unconventional therapies without proper trials or approval is questionable and can be quite dangerous for the patient acutely or in the long term.

Historical aspects and recent advances

In 1962, Landerman observed a lack of kallikrein inhibitory activity in the plasma of HAE patient plasma. The inherited functional deficiency of C1 INH in affected individuals was first identified by Donaldson and Evans in 1963. Replacement therapy with fresh frozen plasma was tried in 1964-65 as the treatment for acute attacks since the patients did not respond satisfactorily to treatment with epinephrine, antihistaminic agents, or corticosteroids. Subsequently, C1 inhibitors purified from human plasma were found to be effective in both prevention as well as in terminating attacks of angioedema when used as the first specific treatment for HAE in 1973. The use of androgens was the first treatment modality tried for abatement for acute attacks. In 1980, Gadek conducted the first well-controlled study for replacement therapy in 8 HAE patients during an HAE attack by using partly purified C1INH from pooled plasma. In this study, 5 out of 8 patients showed abatement of symptoms in addition to increased serum C4 activity. The first C1 esterase inhibitor therapy (plasma derived C1INH concentrate) to be approved by FDA was Cinryze® in 2008. It is pasteurized with an additional nanofiltration step providing additional protection against enveloped and non-enveloped viral particles and prions. In 2009, Berinert® (a pasteurized plasma derived C1INH concentrate) licensed in Europe for over 20 years, got approval from FDA after the completion of a phase III study for use in the treatment of acute

Table 1 Laboratory assessment of HAE

	Anti-genic C1 INH	Functional C1-INH	C4	C1q
Type I HAE	Low	Low	Low	Normal
Type II HAE	Normal-high	Low	Low	Normal
HAE III with normal C1-INH	Normal	Normal	Normal	Normal
Acquired angioedema	Low-normal	Low-normal	Low	Low
ACE inhibitor-induced angioedema	Normal	Normal	Normal	Normal

HAE – hereditary angioedema; C1-INH – C1 inhibitor

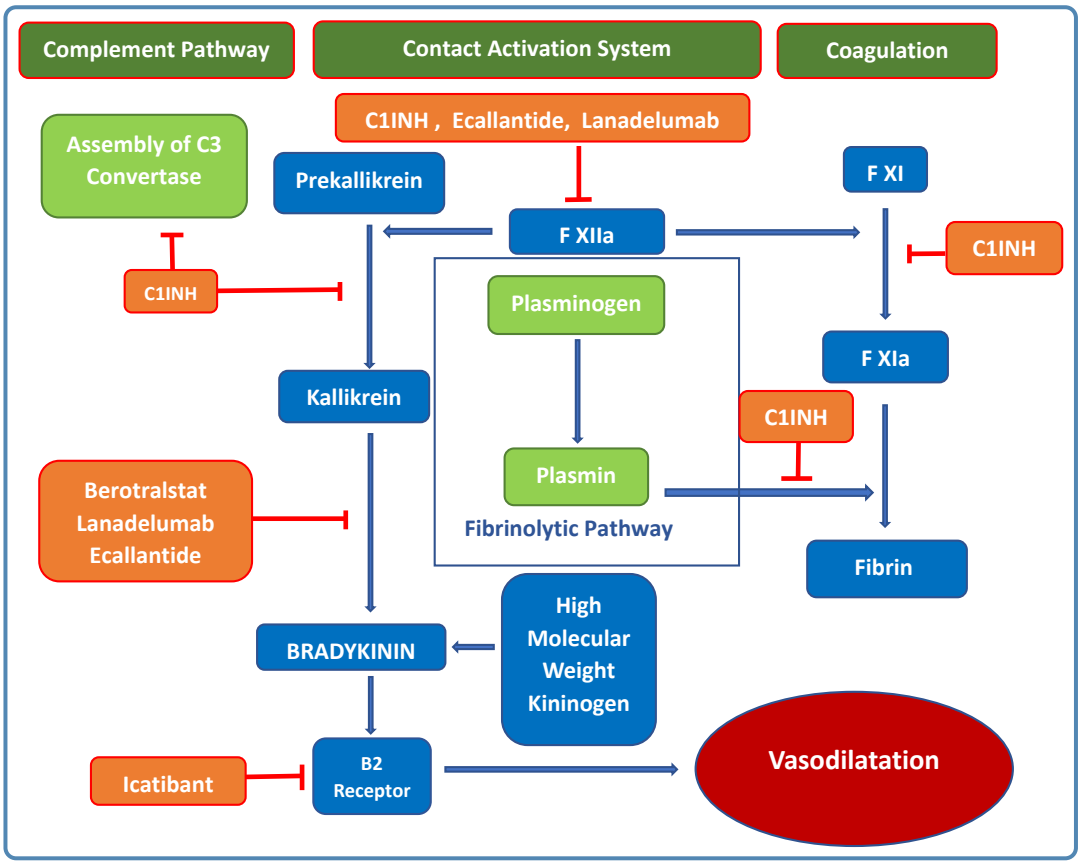

Figure 1 Pathophysiology of hereditary angioedema (HAE) and the site of action of the different drugs used for treatment of HAE. The names of the drugs are mentioned in the orange boxes.

attacks in adolescent and adult patients. Early investigations prepared from the incubation of plasma from HAE patients identified a vascular permeability-enhancing factor named kallikrein assumed to be mediator of swelling in HAE. Trysolol with activity against trypsin, plasmin, and plasma kallikrein was the first plasma kallikrein inhibitor (other than C1INH) to be used. The major drawback associated was severe anaphylactoid reactions. To avoid such adverse effects, a specific plasma kallikrein inhibitor, Ecallantide, FDA-approved in 2009, was used to treat an

angioedema attack with lesser risk of anaphylaxis. Inhibition of the binding of bradykinin to its receptor resolves most attacks of acute attack of HAE. Icatibant, FDA-approved in 2011, acts as a specific and selective competitive antagonist of the bradykinin B2 receptor. It can be self-administered as an on-demand treatment of all types of HAE attacks in adults and children with good safety and tolerability. In 2013, Lanadelumab, a fully human monoclonal antibody inhibitor of plasma kallikrein, got FDA global approval in the USA in 2018 after a succession of trials where it was used as a prophylaxis to prevent HAE attacks in patients aged 12 years or more (Syed et al., 2018).

Current therapies in practice

There are three different principles in the management of HAE. **Table 2** summarizes currently available drugs. Due to easy availability and preventive action on plasminogen activation, Tranexamic acid is also used for acute attacks and prophylactically to treat hereditary angioedema. But the data about efficacy is not convincing.

A. On-demand therapy

On-demand therapy is for the treatment of acute attacks with an FDA-approved on-demand HAE medication (Ecallantide, Icatibant, plasma derived C1-INH, or recombinant C1-INH). The drug becomes effective in 60 min with relief in 2 hours. Second dose may be required. In situations of unavailability of specific therapy, solvent-detergent-treated plasma or FFP can be used along with supportive care (intravenous fluids, antiemetics, narcotic pain medication, or intubation).

B. Short-term prophylaxis

Recommended for all medical, surgical, and dental procedures associated with any mechanical impact to the upper aerodigestive tract. Intravenous plasma-derived C1-INH is the first-line short-term prophylaxis drug for HAE with fresh frozen plasma (FFP) as the second-line agent.

C. Long-term prophylaxis

In hereditary angioedema, long-term prophylaxis is indicated for frequent and/or severe episodes of angioedema.

Management of HAE in the pediatric age group

The first attack of HAE occurs in children before 12 years and 23 years of age in 50% and 90% of cases respectively. Most of the attacks are in the form of angioedema of the skin, may manifest as erythema marginatum in 42%–58% of cases. The frequency and severity of attacks may increase during puberty and adolescence. The earlier the onset of symptoms, the more severe the subsequent course of HAE type 1 or 2. Cases may get critical with delayed diagnosis since abdominal attacks may often go unrecognized. Also, asphyxia can ensue rapidly in children, probably because of the small airway diameter. C1-INH, icatibant and ecallantide are approved on-demand treatments for children with HAE 1 or 2.

Management of HAE in women

The symptoms of HAE are more severe in women. There should be cautious use of exogenous steroids in women. During pregnancy, C1-INH is recommended as first-line therapy for pregnant or breastfeeding HAE-1/2 patients. Short term prophylaxis is indicated for procedures. Ecallantide, Lanadelumab and Berotralstat are not recommended in pregnancy. Androgens are contraindicated. Short-term prophylaxis is not indicated in vaginal delivery, since attacks are uncommon during vaginal delivery. But there is increased angioedema of the vulva after delivery. A dose of plasma derived C1-INH is recommended during vacuum or forceps delivery. A preprocedural dose of plasma derived C1-INH is indicated for planned cesarean delivery. General anesthesia should be given with endotracheal intubation.

Plasma derived C1-INH or recombinant C1-INH for on-demand or prophylaxis is recommended. During lactation, anabolic androgens and tranexamic acid are contraindicated with no safety data on the use of Ecallantide, Icatibant, or Lanadelumab.

Gene therapy and other emerging therapies

Various therapies to cater to the unmet needs of HAE like long duration of action and oral drugs are in various stages of trial. **Table 3** provides

Table 2 Currently Approved Drugs for HAE

Approved drug (Generic name)	Indication	Route	Age of patients	Mechanism	Adverse Effects
Plasma-derived C1-INH	On-demand & routine prophylaxis	Intravenous	≥6 years	Replaces C1-INH	Dysgeusia, injection site reactions, headache, nausea, rash, vomiting, and fever
Recombinant human C1-INH	On-demand & routine prophylaxis	Intravenous	≥12 years	Replaces C1-INH	Anaphylaxis
Ecallantide	On-demand	Subcutaneous	≥12 years	Plasma kallikrein inhibitor	Anaphylaxis
Icatibant	On-demand	Subcutaneous	≥18 years	Bradykinin B2 receptor antagonist	Injection site reaction
Lanadelumab	Routine prophylaxis	Subcutaneous	≥2 years	Monoclonal antibody (plasma kallikrein inhibitor)	Injection site reactions, upper respiratory infections, headache, rash
Berotralstat	Routine prophylaxis	Oral	≥12 years	Plasma kallikrein inhibitor	Abdominal pain, vomiting, diarrhoea

an overview of these emerging therapeutic modalities.

Like all other monogenic disorders, cure by gene therapy is the target of research. Ting Qiu et al in 2019, created a heterozygote C1EI deficient mouse model (S63+/−) that shared characteristics associated with HAE in humans including decreased plasma C1EI and C4 levels with increased vascular permeability of skin and internal organs. Single-time intravenous administration of an adeno-associated virus (AAV) gene transfer vector expressing the genetic sequence of the normal human C1 esterase-inhibitor to the gene-deficient mice resulted in sustained human C1EI activity levels above the predicted therapeutic levels and the correction of the vascular leak in the skin and internal organs.

Another promising gene therapy that is being developed for the treatment of HAE with C1-INH deficiency is BMN331. BMN331 is identified as AAV5 hSERPING1, an adeno-associated virus (AAV5)-based gene therapy vector that expresses wild-type human C1 Esterase Inhibitor (hC1-INH), under the control of a liver-selective promoter. BMN331 is currently under a phase 1 / 2 open-label, dose-escalation study to determine the safety tolerability and efficacy.

Conclusion

Currently available therapies are effective and with improved understanding of pathophysiology, newer modalities are in research mode. C1-INH therapy is now available in India and though the cost is high, it has become accessible for Indian patients through funding support provided by the Government of India under the National Policy for Rare Diseases. Awareness about the disease will help in avoiding misdiagnosis. Gene therapy is an exciting approach with promising results in research studies and this is likely to significantly benefit patients with HAE.

Table 3 Emerging and novel treatments for hereditary angioedema

IONIS-PKKRx	Antisense inhibitor of prekallikrein and bradykinin production: binds and selectively reduces prekallikrein mRNA in the liver (Ferrone et al, 2019).
NTLA-2002	Single-dose therapy development; uses in vivo CRISPR-cas9 genome editing. Designed to inactivate the target gene *KLKB1* to reduce plasma kallikrein activity.
Garadacimab	Monthly Garadacimab (Fully human, IgG4 monoclonal antibody which targets activated FXII) administration was found to be significantly reducing hereditary angioedema attacks in patients aged 12 years and older (Craiget al., 2023).
KVD824	Oral small molecule inhibitor of plasma kallikrein. Phase 2 trial was terminated in October 2022 with high level of liver enzymes cited as the reason.
ALN-F12	Subcutaneously administered Gal-NAc-conjugated siRNA targeting F12 mRNA (ALN-F12) – being done in animal models
PHA-022121	Small molecule bradykinin 2 receptor antagonist (oral). Results of phase 2 trials indicated that effective bradykinin-inhibiting concentrations can be reached within 15 minutes and maintained for at least 10 hours making it ideally suited for single oral dose treatment of acute HAE attacks (Lesage A et al.,2022).

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GeNeViSTA

Next Generation Cytogenetics - Optical Genome Mapping

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Abstract

Optical genome mapping (OGM) is a state-of-the-art technology that is being increasingly adopted by genomic laboratories across the world to detect structural variations (SVs). Conventional cytogenetic/ molecular genetic technologies used to detect SVs like insertions, deletions, inversions, duplications, and translocations have several limitations and/or are highly dependent on the technical expertise of the personnel. Next-generation sequencing which has revolutionized rare disease diagnosis with its ability to detect small genomic variants (such as single nucleotide variants and small indels) also has several shortcomings in identifying structural variations and certain repeat disorders like facioscapulohumeral dystrophy (FSHD). OGM has a significantly higher resolution than techniques such as karyotyping, fluorescence in situ hybridization (FISH) and chromosomal microarray (CMA) and can detect a wider range of variants in a single assay. With a relatively simple workflow and automated analysis pipelines it is less operator dependent and produces robust and reproducible results with rapid turnaround times. Here, we discuss the technology and elucidate its utility in clinical diagnostic settings.

Keywords: Optical genome mapping, cytogenetics, structural variation, genetic testing

Introduction

Structural variants (SVs) play a pathognomonic role in a wide range of genetic diseases. Conventional cytogenetics methods such as karyotyping, fluorescence in situ hybridization (FISH) and chromosomal microarray (CMA) have been at the forefront of standard of care recommendations (SOC) for detection of these variants (Miller et al., 2010). In case of repeat expansion disorders, Southern blotting or polymerase chain reaction (PCR)-based tests have been used for analysis. With the advent of next-generation sequencing (NGS), exome and genome sequencing have been in the spotlight. While these techniques are widely used in clinical laboratories, they are not without limitations. Karyotyping is the gold standard to detect large visible balanced and unbalanced structural variants in addition to aneuploidy. However, the resolution of detection is ~5 to 10 Mb and SVs below this cutoff will be missed. Additionally, there is high variability in results across samples and laboratories, as it is dependent on the expertise of the operator. CMA has an improved resolution of ~ 50kb but cannot detect balanced rearrangements like translocations. FISH is a targeted approach where only a few loci can be assayed at a time. In light of these shortcomings, there exist lacunae for a single technology to accurately detect a variety of structural variants in a short time.

Optical genome mapping – A one stop solution

Optical genome mapping (OGM) is a next generation cytogenomic technique to comprehensively identify all classes of structural variants, copy number variations (CNVs), repeat expansions and contractions, ring chromosomes, absence of heterozygosity (AOH), aneuploidy and triploidy across the whole genome. Depending on the variant type, it offers 100X - 20,000X more resolution than karyotyping (Smith et al., 2022). For OGM on Bionano Saphyr® (Bionano Genomics, San Diego, California, United States), ultra-high molecular weight (UHMW) DNA (≥150 kbp) is isolated from fresh or frozen blood,

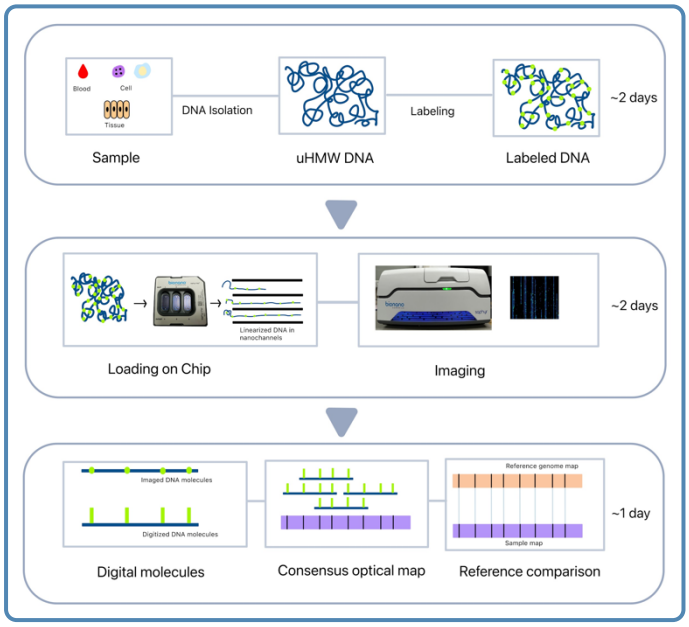

Figure 1 **Optical genome mapping workflow**

Ultra-high molecular weight (uHMW) DNA is isolated from the sample followed by enzymatic labeling with fluorophores. The labeled DNA is loaded onto a Saphyr Chip which linearizes DNA in nanochannels followed by imaging on a Saphyr instrument. The imaged DNA is converted to digitized representations which are then assembled into optical maps. These maps are then compared to the reference genome based on label positions.

bone marrow, tissue, and tumor samples (**Figure 1**). These DNA molecules are then labeled enzymatically with fluorophores using an enzyme DLE-1 at a 6 base-pair sequence motif (CTTAAG) that occurs approximately every 5kb throughout the genome. These labels enable increased resolution and are analogous to bands identified in karyotyping where one band occurs approximately every 5Mb. Three samples can be loaded at once on the Saphyr chip which consists of three flow cells each containing nanochannel arrays within which each single labeled DNA molecule is linearized and imaged on the Saphyr System. Depending on the sample integrity, imaging could take a day or two. The images consist of a unique pattern of bands represented by fluorescent labels which are further converted into digital representations called optical maps and the same can be analyzed bioinformatically. The labels in the optical maps only represent known physical locations of the motif sequence and cannot be used to interrogate regions at single base pair resolution. The optical maps from the sample are mapped to an *in silico* labeled reference genome to detect mismatches in the label patterns. These patterns (**Figure 2**) can then be used to detect structural variants for example, extra labels at a particular locus in the sample when compared to reference could indicate an insertion, missing labels indicate a deletion, and a set of repeated labels could indicate a duplication.

In terms of the bioinformatics workflow, only DNA molecules ≥150 kb are used for analysis. Depending on the SV detection type, there is a choice of four pipelines. For the detection of germline SVs, a *de novo* assembly pipeline can be chosen. In case of somatic detection, the rare variant pipeline can be applied. For targeted approaches encompassing repeat contractions, in case of facioscapulohumeral muscular dystrophy (FSHD) and repeat expansions such as fragile X syndrome, there are specialized EnFocus analysis being offered. OGM can detect SV classes from 500 bp onwards which makes it highly sensitive when compared to other methods. The processing time for the pipelines is very fast when compared to existing methodologies. For our in-house data, the *de novo* assembly pipeline took approximately 12 hours for workflow completion whereas for EnFocus for FSHD it was 8 hours

on our computer servers. We estimate that the end-to-end processing for a clinical sample from DNA extraction to clinical results would take less than a week's time using OGM.

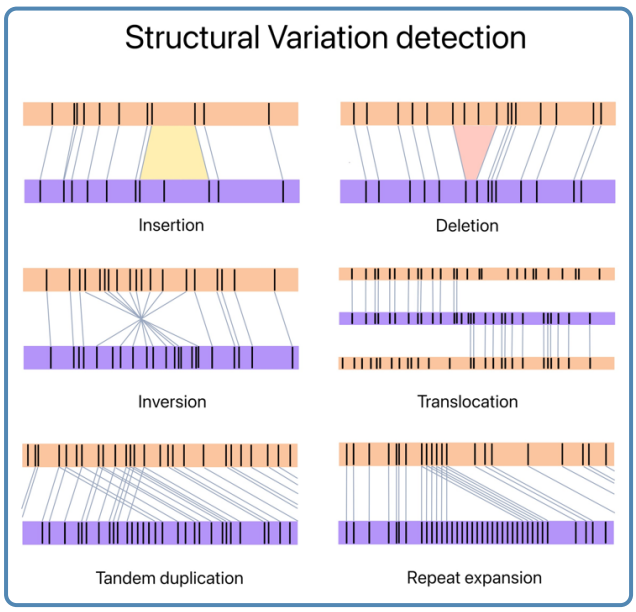

Figure 2 **Structural variant detection**
SVs can be detected by comparing the label patterns between the reference genome (pink) and the sample (purple). In the examples shown, additional labels indicate an insertion, missing labels indicate deletion, inverted label orientation are characteristic of inversions, a single map mapping to distant regions of the genome indicates a translocation and based on the repeating patterns of labels duplications and repeat expansions can be detected. Various other types of complex variants not seen in the above depictions can also be identified by optical genome mapping.

Optical genome mapping versus current standard of care methods

OGM has been assessed in comparison to the current SOC methods in multisite studies involving prospective and retrospective constitutional cohorts, and prenatal and postnatal cohorts. The studies interrogated concordance in technologies across neurodevelopmental problems [autism spectrum disorder (ASD), intellectual disability/ developmental delay (ID/DD), attention-deficit hyperactivity disorder/ oppositional defiant disorder (ADHD/ ODD)], chromosomal aberrations, fragile X syndrome and FSHD (Mantere et al., 2021; Broeckel et al., 2022; Stevenson et al., 2022; Iqbal et al., 2023). Concordance was found ranging from > 98% to 100% depending on the type of anomaly. In addition, several OGM exclusive SVs were also found which were missed out in current SOC methods (Broeckel et al., 2022). The utility of OGM is also being increasingly realized in hematological malignancies where chromosomal abnormalities play a defining role in prognostication of the disease (Neveling et al., 2021; Gerding et al., 2022).

Illustrative clinical scenarios

Patient 1: A 9-year-old male child, born to non-consanguineous parents with severe wasting and weakness of shoulder and arm muscles and abnormal gait was noted to have bilateral facial muscle weakness and scapular winging. There was no significant family history. With a provisional diagnosis of FSHD, his blood sample was analyzed for repeat contraction at D4Z4 locus on chromosome 4 by OGM. After preparation and quality control (QC) checks the sample was run on Bionano Saphyr and the data generated was analyzed using the EnFocus FSHD pipeline, a targeted approach to analyze specific regions on chr4 and chr10 since both contain the D4Z4 arrays. This analysis also enables identification of both permissible and non-permissible haplotypes along with estimation of D4Z4 repeat counts accurately on both the chromosomes. Here, we were able to identify a repeat contraction in the D4Z4 repeat array on 4q35 containing 3 repeats (normal >11) along with the presence of permissive haplotype 4qA **(Figure 3)**. This confirmed the disease diagnosis and the short repeat size detected partly explains the severity of the disease in this child.

Case 2: A 3-year-old female child with developmental delay, seizures and dysmorphism had additional material on p arm of chromosome 12 on karyotyping. On OGM the child was identified to have insertion of duplicated genomic material of ~16Mb from 12q24.21 at 12p13.33 (**Figure 4**). The rest of the CNVs identified were not pathogenic. Thus, OGM was not only able to recognize the chromosomal abnormality but also identified its constitution. This enables accurate genetic diagnosis for better prognostication and

management of such children.

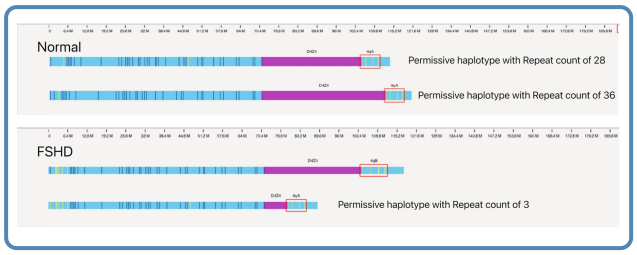

Figure 3 **Identification of D4Z4 repeat contraction in facioscapulohumeral muscular dystrophy**
The first panel shows a sample containing the 4qA permissive haplotype along with a D4Z4 repeat array indicating a normal range. The second panel shows a severe case of FSHD indicated by the permissive haplotype and a repeat contraction indicating only presence of three D4Z4 repeat units.

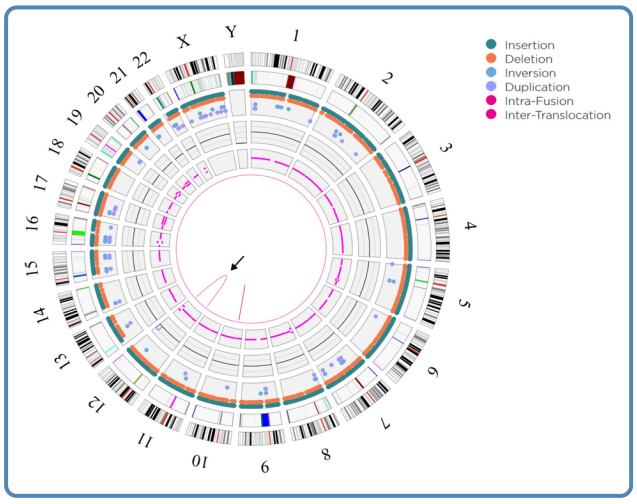

Figure 4 **Circos plot showing translocation and other genome-wide structural variants**
The insertion of material from 12q at 12p is visualized as an arc within chromosome 12 as indicated by an arrow. CNV analysis is suggestive of copy number gain at distal part of 12q (exact loci are evident in a different view not shown in this picture)

Limitations

Extraction of uHMW DNA is an essential requirement necessitating the transport of freshly drawn blood samples to the testing laboratory within 48-72 hrs. This also means that old samples/ stored DNA extracted through conventional methods cannot be used. OGM cannot detect SVs in the centromeric and the telomeric regions. Since it cannot detect acrocentric short arm fusions it cannot distinguish, for example, between a free trisomy or an unbalanced Robertsonian translocation. The technology is relatively expensive, especially in the context of developing countries.

Overall, OGM has been a positive and much needed development in the field of molecular cytogenetics. It has the potential to replace conventional testing methodologies in clinical diagnostics and hopefully, wider adaptation will make the technology more affordable.

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GeNeXpreSS

Utility of Long-read Sequencing in Human Genetic Disorders

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Cytogenomic characterization of a novel de novo balanced reciprocal translocation t(1;12) by genome sequencing leading to fusion gene formation of EYA3/EFCAB4b (Dutta et al., 2022)

Disease-associated structural variants (SV) often have breakpoints within the gene or in its vicinity. The accurate detection of breakpoints helps in identifying the molecular mechanism and the risks involved with the disrupted genes. The detection of SVs is mostly done by chromosomal analysis, but karyotyping is a low-resolution technique. In this study, the authors have evaluated a girl with short stature wherein the cytogenetic analysis revealed an additional material on the 12p region and fluorescence *in situ* hybridization (FISH) with whole chromosome paint (WCP) FISH probe for chromosome 12 revealed a translocation of t(1;12)(p36.1;p13.32). The parents did not have the translocation; hence it was most likely of *de novo* origin. Microarray comparative genomic hybridization (CGH) ruled out significant copy number variations. The physical mapping with FISH clones identified the spilt signals. Long-read sequencing by Oxford Nanopore Technologies was done and an efficient pipeline was developed which helped in the identification of 4 unique chimeric reads. Sanger sequencing confirmed the junction fragment, revealing microhomology-mediated break-induced repair (MMBIR). The breakpoint on chromosomes 1 and 12 was found to disrupt *EYA3* and *EFCAB4B* genes. Real time PCR showed an over-expression of both genes due to fusion gene formation. This study highlights the utility of long-read sequencing in delineating breakpoints of balanced reciprocal translocations and easy development of the pipelines.

Identification and characterization of two DMD pedigrees with large inversion mutations based on a long-read sequencing pipeline (Geng et al., 2023)

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder. The mutation spectrum includes 60-70% of deletion/duplications, 20% of point mutations, and rarely structural variations like translocations and inversions. Most of the deletion/duplications can be detected by multiplex ligation-dependent probe amplification (MLPA) and sequence variants/ point mutations using Sanger sequencing or short-read next-generation sequencing (NGS). In this report, two cases of DMD were studied wherein no deletion/duplication or point mutation was identified. First was a 5-year-old boy with family history of DMD. His serum creatine kinase (CPK) levels were high, and muscle biopsy showed absence of dystrophin. MLPA could not detect any copy number variations (CNVs) and exome sequencing did not detect any sequence variants. In view of the clinical phenotype being strongly suggestive of DMD, a rare mutation was suspected. Chromosomal analysis showed normal karyotype of 46, XY. RNAseq and cDNA array capture sequencing showed absence of 3-55 exons hinting at a rare mutation. Optical genome mapping revealed a 55 Mb pericentric inversion at Xp21 to Xq21 region. Long-read sequencing was performed by Oxford Nanopore sequencing and SMRT technology of PacBio, and the exact breakpoint region was identified. The breakpoint region showed a 16 base pair insertion and repeat elements. Induced pluripotent stem cells iPSC and full cDNA sequence study revealed 4 transcripts, one was small with exons 1 and 2 and the other 3 were fusion transcripts, formed as a result of inversion. Sanger confirmation was done in several family members and carrier analysis of a

pregnant cousin was normal. The second case was a 10-year-old boy with no family history but incidentally found to have elevated serum CPK levels. Immunohistochemistry (IHC) of muscle biopsy showed absence of dystrophin staining. Direct long-read sequencing identified a 96 Mb inversion. Both the breakpoint regions anchored several repeat elements. The authors were able to establish an efficient long-read sequencing pipeline for inversion detection.

Comprehensive genetic diagnosis of tandem repeat expansion disorders with programmable targeted nanopore sequencing (Stevanovski et al., 2022)

Short tandem repeats (STR) are 2-6 base pair repeats of DNA. Usually long expanded STR alleles are pathogenic and more than 40 heritable disorders like Huntington disease, Fragile X syndrome, Myotonic dystrophy, etc. are known to be caused by this mechanism. This study demonstrates the validity and utility of programmable targeted Oxford nanopore sequencing for the genetic diagnosis of STR expansion disorders. Targeted long-read sequencing is one of the techniques used by Oxford Nanopore Technologies (ONTs) using "Read Until" functionality. The ONT device is programmed to recognize and reject/accept specific target selections. It can be used to achieve accurate molecular characterization of all known neuropathogenic STRs in a single assay. The authors customized a panel with all known STRs in a single assay primarily for neurological and neuromuscular diseases. For each gene, the entire locus was targeted including 50 kb of flanking sequences in either direction. Readfish software was used to target sequences of 37 DNA samples from patients and observed a consistent reduction in read length for off-target reads when compared to on-target reads. Although this study could identify the tandem repeat expansion disorders it is still considered to be a research tool.

Detecting cell–of–origin and cancer–specific methylation features of cell–free DNA from Nanopore sequencing (Katsman et al., 2022)

Circulating cell-free DNA (cfDNA) can reveal informative features of its tissue of origin, including somatic genome alterations, DNA modifications, and cell type-specific fragmentation patterns. ONT can call accurate DNA methylation from native DNA and produce single base-pair resolution results highly similar to bisulfite sequencing. This study shows that cell type and cancer-specific methylation can also be detected, as well as cancer-associated fragmentation signatures. This study also highlights that ONT's shallow whole-genome sequencing (WGS) could be a powerful tool for liquid biopsy. In this study, the cell type fractions from cfNano were estimated. The sample size was small but their results suggest that cancer-specific features of DNA methylation, and fragmentation were concordant between cfNano and Illumina-based WGS methods. Also, the results suggest that short di-nucleosomes could be a more robust cancer marker than short mono-nucleosomes, although this needs to be validated in a larger study. This study showed the feasibility of ONT sequencing for circulating tumor DNA detection by comparing methylation and several fragmentation features to matched Illumina samples and comparable Illumina-based datasets.

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8th Annual Conference of the
Society for Indian Academy of Medical Genetics

IAMG 2023

30th November, 2023 | **1st - 3rd December, 2023**

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Organized by: Institute of Medical Genetics & Genomics, Sir Ganga Ram Hospital, New Delhi

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Conference Highlights

- *Unravelling the genome beyond "exome only approach"*
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- *Autism spectrum disorder - genetics and treatment*
- *Cancer Genomics*

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Richard Boles , USA

and many more National and International Faculty

Scan QR Code To Register

Pre Conference Workshops

Pre Lunch Workshop
9.00 am - 1.00 pm

Workshop 1

A Hands on Workshop on Whole Genome Sequencing - Hits and Misses

Dr David Adams, USA & Dr Madhuri Hegde, USA

Post Lunch Workshop
2.00 pm - 5.00 pm

Workshop 2

Autism and ADHD: Latest Science in Genetics and Mitochondrial Medicine & Improved Outcomes

Dr Richard Boles, USA

Any 1 Workshop
INR 3000/-

For Both Workshops
INR 4500/-

Conference Registration Details

Fees Inclusive of 18% GST

Category	Early Bird Till 14th September 2023	From 15th September - 29th November	From 30th November Onwards / Onspot
SIAMG Member	INR 6000/-	INR 7000/-	INR 10000/-
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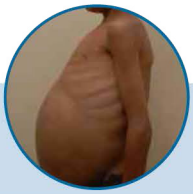

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