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Review Article

A genomic perspective of benign hematological disorders in the era of next-generation sequencing

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ABSTRACT

Next-generation sequencing (NGS) has revolutionized the diagnostic approach to hematological disorders, enabling precise identification of genetic alterations that underlie both benign and malignant conditions. By detecting single-nucleotide variants, insertions/deletions, structural rearrangements, and copy number changes, NGS provides unparalleled diagnostic accuracy. This technology encompasses various techniques, including targeted sequencing, whole exome sequencing (WES), whole genome sequencing (WGS), and transcriptome analysis, each suited to specific clinical applications. Targeted NGS focuses on clinically relevant genes, offering a cost-effective approach for diseases with established genetic bases, while WES and WGS allow for broader analyses to uncover novel mutations in cases of uncertain genetic etiology. NGS surpasses single-gene testing by addressing the lack of specificity in clinical and laboratory features, facilitating the analysis of multiple candidate genes simultaneously. Studies highlight that relying solely on phenotype and traditional methods can result in misdiagnoses in 10–40% of cases, leading to inadequate treatment and increased patient burden. NGS mitigates these risks by providing a comprehensive and efficient diagnostic strategy. Despite its advantages, NGS interpretation poses challenges, particularly in classifying variants according to the American College of Medical Genetics and Genomics guidelines. Variants are categorized into five classes, ranging from pathogenic to benign, with many novel variants remaining of uncertain significance. Advanced tools and population databases, such as SIFT, PolyPhen-2, and ExAC, aid in variant assessment. This review underscores the transformative role of NGS in diagnosing inherited benign hematological disorders. By improving diagnostic precision, understanding disease mechanisms, and enabling genetic counseling, NGS fosters the advancement of personalized medicine. Its integration into routine diagnostics enhances cost-effectiveness and expands the potential for updating targeted panels as new genes are discovered, solidifying its pivotal role in modern healthcare.

Keywords: Benign hematology, Exome sequencing, Genomics, Molecular hematology, Next-generation sequencing

INTRODUCTION

Next-generation sequencing (NGS) has transformed the diagnostic landscape of hematological disorders by enabling the comprehensive detection of genetic alterations associated with both benign and malignant conditions. Its ability to identify single-nucleotide variants, insertions or deletions (in-dels), complex structural rearrangements, and copy number changes provides unparalleled diagnostic precision. NGS encompasses various techniques, such as targeted sequencing, whole exome sequencing (WES), whole genome sequencing (WGS), and transcriptome analysis. Each approach is tailored to specific clinical needs.[1]

Targeted NGS (T-NGS) focuses on preselected clinically relevant genes, offering a cost-effective solution for diseases with known genetic etiologies. WES sequences approximately 30,000 genes and is suitable for conditions with unclear genetic origins, while WGS provides a comprehensive analysis of coding and non-coding regions, allowing the discovery of novel mutations. The choice of method depends on the nature of the disease. For inherited or congenital conditions, germline mutations are typically analyzed, while acquired disorders often involve somatic mutations. This differentiation ensures that the testing strategy aligns with the underlying pathophysiology of the disease.[2]

The advantages of using NGS over single-gene testing extend beyond cost-effectiveness. Clinical and laboratory features often lack specificity for a single condition, necessitating the analysis of many candidate genes before arriving at a diagnosis. Studies indicate that in 10–40% of cases, misdiagnosis or lack of diagnosis occurs when relying solely on phenotype and traditional non-NGS testing. Such misdiagnoses can lead to incorrect or inadequate treatment, causing undue anxiety, impairing quality of life, and increasing costs.[2]

A critical challenge in the NGS approach lies in the interpretation of variants. Identified variants are classified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG). Variants are scored on a five-class system: pathogenic (class 5), probably pathogenic (class 4), uncertain significance (class 3), probably benign (class 2), or benign (class 1). Pathogenic variants include nonsense mutations, frameshifts, canonical splice site alterations, and significant deletions. Variants such as missense mutations, in-frame in-dels, and intronic or promoter variants require additional evaluation, including allele frequency in population databases (e.g., ExAC and dbSNP), published literature, segregation studies, family history, and in silico predictions from tools such as SIFT, PolyPhen-2, and MaxEntScan. Most novel sequence variants without functional evidence are categorized as variants of uncertain significance (class 3).[3]

This review classifies the inherited benign hematological disorders, highlighting the utility of NGS in diagnosing these conditions. It is important to note that the spectrum of diseases is vast, and this review is by no means exhaustive. NGS provides an invaluable tool for improving diagnosis, understanding disease mechanisms, and guiding genetic counseling, ultimately advancing personalized care in benign hematological disorders.

THALASSEMIA AND HEMOGLOBINOPATHIES

Routine molecular testing for the genetic diagnosis of thalassemia has traditionally relied on Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA). While these methods remain effective, comprehensive screening strategies are increasingly recognized as essential for the management and prevention of thalassemia. Many clinical and research laboratories have begun adopting NGS technologies due to their efficiency, versatility, and cost-effectiveness. However, the application of NGS in thalassemia is still in its early stages, driven by the need for alternative DNA screening tools.

T-NGS panels often exclude alpha globin genes (*HBA1* and *HBA2*) due to several challenges:

1. High sequence homology: The extensive similarity between *HBA1* and *HBA2* sequences complicates analysis, increasing the likelihood of false-positive results.
2. Copy number variations (CNVs): Pathogenic variants in globin genes frequently involve CNVs, such as large deletions (e.g., α3.7 deletion) or gene multiplications, which are challenging for NGS to detect accurately.
3. Deep intronic mutations: These mutations may not be captured during exome sequencing, leaving gaps in diagnostic capability.

For most cases of thalassemia and hemoglobinopathies, clinical and laboratory findings provide strong diagnostic clues, often making extensive genetic testing unnecessary. In such situations, conventional molecular techniques like Sanger sequencing are sufficient for identifying point mutations and small in-dels. For larger insertions, deletions, or CNVs, robust methods such as gap-polymerase chain reaction (PCR) or MLPA are preferred.

While NGS demonstrates superiority in detecting rare variants, resolving complex hematological cases, and offering non-invasive alternatives for neonatal diagnosis, its limitations prevent it from replacing traditional methods as a stand-alone tool. Nonetheless, as technology advances, NGS is poised to play an increasingly significant role in the genetic analysis of thalassemia.

HEMOLYTIC ANEMIAS

NGS has significantly advanced the diagnosis of hereditary hemolytic anemias (HHAs), a diverse group of disorders characterized by premature red blood cell destruction. Traditional diagnostic methods, including clinical evaluation and biochemical assays, often fall short in pinpointing the exact genetic causes of HHAs due to their genetic heterogeneity. NGS addresses this challenge by enabling the simultaneous analysis of multiple genes associated with these conditions.

Studies have demonstrated the efficacy of NGS in diagnosing unexplained hemolytic anemias, with success rates ranging from 39% to 70%.[4]

This high diagnostic yield underscores NGS's capability to detect both single nucleotide variants and CNVs across a

broad spectrum of genes. For instance, the HHA gene panel offered by Mayo Clinic Laboratories utilizes NGS to assess 37 genes linked to these disorders, facilitating comprehensive genetic evaluations for patients with personal or family histories suggestive of HHAs.

The advantages of NGS over traditional single-gene testing are notable. NGS is cost-effective and particularly beneficial when clinical and laboratory features are not specific to a particular condition, necessitating the analysis of multiple candidate genes to establish a diagnosis. In fact, studies have shown that relying solely on phenotype and traditional non-NGS testing can lead to misdiagnosis or no diagnosis in 10–40% of cases. This can result in incorrect or inadequate treatment, causing anxiety and adversely affecting quality of life.

However, the implementation of NGS in clinical practice is not without challenges. Interpreting identified variants requires adherence to guidelines, such as those from the ACMG, which classify variants based on pathogenicity. In addition, the high level of gene homology, presence of CNVs, and deep intronic mutations can complicate NGS analysis in these disorders.[5]

Despite these challenges, NGS has proven invaluable in identifying rare variants, resolving complex hematological cases, and offering non-invasive alternatives for neonatal diagnosis. Its comprehensive approach enhances diagnostic accuracy, informs clinical management, and enables genetic counseling for affected individuals and their families. As technology advances and our understanding of the genetic underpinnings of HHAs deepens, NGS is poised to play an increasingly pivotal role in the diagnosis and management of HHAs.

IRON-RELATED DISORDERS

Iron-related disorders encompass a spectrum of conditions that arise from disruptions in iron metabolism, transport, or regulation. Cellular iron homeostasis is regulated by two RNA-binding proteins: Iron regulatory protein 1 and 2 (IRP1 and IRP2). Another important liver hormone which regulates iron availability is Hepcidin.[6] NGS has significantly enhanced the diagnosis and understanding of these disorders by identifying key genetic mutations and their functional impact. The following entities fall under this category:

Hepcidin disorders

Hepcidin disorders constitute either hepcidin deficiency or hepcidin excess.

Hereditary hemochromatosis (HH): (Hepcidin deficiency)

HH is a condition characterized by iron overload due to dysregulated intestinal absorption of iron. Based on the gene defect, HH was formerly classified into 4 types, with type 2 further subdivided into subtypes A and B [Table 1].

HFE is the most common gene associated with HH, particularly the C282Y and H63D mutations. Hemochromatosis (HC) classification has now been revised by the BIOIRON Society because of a few caveats in the former classification.[7]

Some cases show a digenic inheritance deriving from the combination of pathogenic variants in 2 different genes involved in iron metabolism (e.g., single p.Cys282Tyr + heterozygous variants in hemojuvelin [HJV], HAMP, or transferrin receptor 2 [TFR2]).

Some cases do not display variants in any of the 5 classical HC genes (i.e., HFE, HAMP, HJV, TFR2, and SLC40A1). Recently, a small case series have shown variants in the BMP6 gene to be associated with HH. BMP6 encodes one of the major activators of hepcidin expression in response to iron. The role of such variants is still controversial; nonetheless, they broaden the spectrum of genetic defects potentially responsible for HH.

The new classification of HH proposed by the working group is shown in Table 2.[7]

Table 1: Former classification of hereditary hemochromatosis

Classifications	Gene involved	Inheritance
Type 1	HFE (Hereditary hemochromatosis)	AR
Type 2A	HJV (Hemojuvelin)	AR
Type 2B	HAMP (Hepcidin)	AR
Type 3	TFR2 (Transferrin receptor2)	AR
Type 4	SLC40A1 (Ferroportin)	AD

AR: Autosomal recessive, AD: Autosomal dominant

Table 2: New classification of hereditary hemochromatosis as recommended by the BIOIRON society.

Novel classification	Molecular pattern
HFE related	p.Cys282Tyr homozygosity or compound heterozygosity of p.Cys282Tyr with other rare HFE pathogenic variants, or rarely HFE deletion
Non-HFE related	Rare pathogenic variants in "non-HFE" genes: HJV related HAMP related TFR2 related SLC40A1 (GOF) related
Digenic	Double heterozygosity and/or double homozygosity/heterozygosity for mutations in 2 different genes involved in iron metabolism (HFE and/or non-HFE)
Molecularly undefined	Molecular characterization (still) not available after sequencing of known genes (provisional diagnosis)

TFR2: Transferrin receptor 2, HJV: Hemojuvelin, HAMP: Hepcidin antimicrobial peptide, GOF: Gain of function

Iron-refractory iron deficiency anemia (IRIDA)

IRIDA is caused by mutations in the *TMPRSS6* gene, which encodes matriptase-2, a regulator of hepcidin, leading to inappropriate hepcidin overproduction, reduced iron absorption, and poor response to oral iron therapy.[8]

Disorders of iron transport, utilization, and recycling

Congenital hypotransferrinemia

This rare disorder is caused by mutations in the *TF* gene, leading to insufficient transferrin and impaired iron transport to cells, causing severe anemia with iron overload in non-hematopoietic tissues.[9]

Ferroportin disease

Mutations in the *SLC40A1* gene impair iron export from macrophages and enterocytes, leading to iron sequestration and anemia.[10]

Sideroblastic anemia

Sideroblastic anemia is characterized by defective heme synthesis, resulting in mitochondrial iron accumulation in erythroid precursors and microcytic hypochromic anemia. Mutations in genes such as *ALAS2*, *SLC25A38*, and *GLRX5* are implicated.

Aceruloplasminemia

Aceruloplasminemia is a rare autosomal recessive disorder caused by mutations in the *CP* gene, leading to absent ceruloplasmin activity, impaired iron metabolism, and iron accumulation in tissues. It presents with neurological symptoms, diabetes, and anemia due to iron overload.

Isolated hyperferritinemia

Isolated hyperferritinemia refers to elevated serum ferritin levels without evidence of systemic iron overload or inflammation. It is commonly associated with hereditary hyperferritinemia-cataract syndrome due to mutations in the *ferritin light chain* gene or can result from metabolic conditions like fatty liver or alcohol use.[10]

Porphyria

Porphyrias are a group of disorders caused by defects in heme biosynthesis enzymes. NGS panels targeting genes such as HMBS, PPOX, and CPOX have improved the diagnosis of conditions such as acute intermittent porphyria and erythropoietic protoporphyria.

HEREDITARY ERYTHROCYTOSIS

Molecular analysis plays a pivotal role in the diagnosis and understanding of hereditary erythrocytosis, a rare condition characterized by elevated red blood cell mass due to genetic mutations. Unlike secondary erythrocytosis caused by external factors such as hypoxia or tumors, hereditary forms arise from mutations in genes regulating oxygen sensing, erythropoiesis, or hemoglobin affinity. Commonly implicated genes include *erythropoietin receptor* (*EPOR*), *von Hippel-Lindau* (*VHL*), *prolyl hydroxylase domain-containing protein 2* (*PHD2/EGLN1*), and *hypoxia-inducible factor 2-alpha* (*HIF2A/EPAS1*). Molecular testing, typically using techniques such as targeted gene panels or WES, enables the precise identification of these mutations.[11]

Such analyses are critical for differentiating hereditary erythrocytosis from acquired causes like polycythemia vera, which involves JAK2 mutations. The identification of pathogenic variants provides insights into the underlying mechanism—whether it is due to increased sensitivity to erythropoietin, disrupted oxygen-sensing pathways, or high-oxygen-affinity hemoglobins. Molecular testing also facilitates early diagnosis, family screening, and tailored management strategies. For instance, individuals with high oxygen-affinity hemoglobin variants may require different interventions compared to those with mutations in oxygen-sensing genes. Overall, molecular analysis not only refines diagnostic accuracy but also guides personalized treatment and improves the long-term prognosis of patients with hereditary erythrocytosis.[12]

Classification of hereditary erythrocytosis based on gene mutation:

- Type1: *EPOR*
- Type2: *VHL*
- TYpe3: *EGLN1* (*PHD2*)
- Type4: *EPAS1* (*HIF2A*)
- Type5: *EPO*
- Type6: *HBB*
- Type7: *HBA1*, *HBA2*
- Type8: *BPGM*

INHERITED BONE MARROW FAILURE SYNDROME (BMFS)

BMFS often present with at least one extra-hematopoietic manifestation. The diagnosis of an inherited BMFS should be considered in pediatric patients when at least one extra-hematopoietic feature associated with BMFS is identified, either clinically or through investigations. However, the absence of such features does not rule out the possibility of BMFS. Therefore, BMFS should also be considered in

children presenting with isolated aplastic anemia (AA), myelodysplastic syndrome (MDS), or leukemia.[13]

In all children with AA or MDS, particularly in the presence of monosomy 7, an additional chromosome 3, or a complex karyotype, testing for Fanconi anemia (FA) is recommended. Initial testing involves stress cytogenetics using agents such as diepoxybutane or mitomycin C. However, these tests have limitations. For example, somatic mosaicism may lead to false-negative results, and other diseases, such as Nijmegen breakage syndrome, may produce false-positive findings in chromosome breakage studies.

Inherited BMFS encompasses a broad spectrum of diseases, requiring extensive gene panel coverage when performing NGS. Occasionally, a novel or uncertain variant in a known disease-associated gene may be identified. In such cases, segregation analysis within the family and functional studies are essential to confirm whether the variant is responsible for the disease.

Why is it important to rule out IBMFS in cases of pancytopenia/AA?[13]

i. Risk of Complications: IBMFS syndromes often predispose patients to long-term complications such as cancer (e.g., leukemia or solid tumors). Identifying the inherited nature of the disease enables close monitoring and early intervention for malignancies.
ii. Tailored Management and Treatment: IBMFS often requires specific management strategies that differ from acquired AA. For instance, patients with FA may not tolerate standard immunosuppressive therapies used for acquired cases and instead benefit from early hematopoietic stem cell transplantation (HSCT).
iii. Patients with FA or DC are more prone to liver disease and heightened drug sensitivity, necessitating careful monitoring, especially after allogeneic HSCT.
iv. Family Screening and Genetic Counseling: Since IBMFS syndromes are inherited, diagnosing an underlying genetic cause allows for family member testing to identify carriers or asymptomatic individuals who may also require monitoring or treatment.
v. Donor Selection for HSCT: In cases where HSCT is required, ruling out IBMFS is critical for identifying suitable donors. A related donor carrying the same genetic defect could compromise the success of the transplant.

CONGENITAL NEUTROPENIA (CN)

CN is a group of disorders characterized by three main features: reduced neutrophil counts, predisposing patients to recurrent infections, various organ dysfunctions, and high risk of leukemic transformation. There are about 24 genes which are responsible for this syndrome. NGS has been instrumental in identifying causative mutations in genes such as *ELANE*, *G6PC3*, *HAX1*, and *CXCR4*. Even after analysis based on T-NGS, about 40% of CN remain without a molecular etiology.[14] Such cases can be resolved using WES technology where novel causative genes involved in rare forms of syndromic CN can be discovered.

Mutations in some genes such as *GATA2* and *CSF3R* can be either germline or somatic. The germline status can be confirmed by analyzing DNA extracted from skin fibroblasts, nails, or hair follicles.[14]

Cyclic neutropenia, a type of severe CN, is an autosomal dominant disorder characterized by fluctuating blood neutrophil count, usually with a 21-day periodicity. The most common molecular defect lies in the *ELANE* gene.[15]

COAGULATION AND BLEEDING DISORDERS

Molecular analysis has transformed the diagnostic and therapeutic landscape of coagulation and bleeding disorders, a diverse group of conditions caused by abnormalities in clotting factor production, function, or regulation. These disorders include hemophilia A and B, von Willebrand disease (VWD), and rare clotting factor deficiencies. Genetic mutations in key coagulation-related genes, such as *F8*, *F9*, and *VWF*, are often the underlying cause. Identifying these mutations through molecular techniques provides definitive diagnoses, helps predict disease severity, and informs treatment strategies.[16]

NGS has become a cornerstone in the molecular investigation of these disorders. NGS panels can simultaneously analyze multiple genes involved in coagulation pathways, offering a comprehensive approach to diagnosing complex or atypical presentations.

For example, in hemophilia A, the F8 gene commonly exhibits large intron inversions, point mutations, and deletions. Intron 22 and intron 1 inversions are specifically detected using inverse PCR. If these inversions are absent, sequencing the entire F8 gene is necessary. However, given the large size of the F8 gene with its 28 exons, Sanger sequencing is labor-intensive and cost-prohibitive, making NGS an essential tool for pinpointing point mutations. Similarly, in VWD (VWD), sequencing the VWF gene can identify mutations affecting factor production, multimerization, or clearance.[16,17]

Beyond identifying causative mutations, molecular analysis also aids in understanding genotype-phenotype correlations, particularly in conditions with variable expressivity, such as VWD. It enables personalized medicine approaches, such as predicting inhibitor development in hemophilia patients or tailoring replacement therapy to specific genetic variants. Moreover, molecular studies play a vital role in family screening and genetic counseling, carrier screening, and aiding in prenatal decision-making.

Emerging technologies, such as *clustered regularly interspaced short palindromic repeat*-based gene editing, gene therapy, and RNA therapeutics, have further underscored the importance of molecular insights in bleeding disorders, opening avenues for curative therapies.[18]

INHERITED THROMBOCYTOPENIA

Molecular analysis plays a pivotal role in the diagnosis and understanding of inherited thrombocytopenia, a heterogeneous group of disorders characterized by low platelet counts due to genetic abnormalities. Inherited thrombocytopenia can result from mutations in a wide array of genes that regulate platelet production, maturation, or function.[19]

Commonly implicated genes include *MYH9*, associated with *MYH9*-related disorders; thrombocytopenia-absent radius syndrome, congenital amegakaryocytic thrombocytopenia, *RUNX1*, linked to familial platelet disorder with a predisposition to acute myeloid leukemia; and *ANKRD26* and *ETV6*, both associated with thrombocytopenia and increased malignancy risk.[20] Advanced molecular techniques, such as NGS, allow for comprehensive evaluation of these genes, identifying both common and rare mutations that contribute to the condition.

NGS panels designed for thrombocytopenia target multiple genes simultaneously, enabling rapid and cost-effective identification of causative variants. For cases with atypical presentations or unclear inheritance patterns, WES can uncover novel mutations and non-coding region variants that might be missed by targeted panels. Functional assays and gene expression studies often complement molecular findings to confirm pathogenicity.[21]

Molecular analysis not only establishes a definitive diagnosis but also helps classify thrombocytopenia into syndromic or non-syndromic forms, influencing clinical management and genetic counseling. It aids in risk stratification for associated complications, such as bleeding tendencies, leukemic transformation, or skeletal abnormalities in syndromic cases. By analyzing genes such as *MYH9*, *MPL*, and *RBM8A*, *NGS* clarifies genetic causes, enabling differentiation from acquired thrombocytopenia and informing treatment decisions such as platelet transfusion or transplantation.

THROMBOPHILIA

Thrombophilia refers to a predisposition to abnormal blood clot formation due to genetic, acquired, or mixed factors. Common genetic causes of inherited thrombophilia include Factor V Leiden (FVL) (F5) mutation, prothrombin (F2) gene mutation, and deficiencies in natural anticoagulants such as protein C, protein S, and antithrombin. Molecular testing enables the identification of these mutations and clarifies ambiguous clinical presentations.[21]

The FVL mutation, caused by a single nucleotide polymorphism (c.1691G>A), results in resistance to activated protein C and is the most common inherited thrombophilia. Similarly, the prothrombin G20210A mutation increases plasma prothrombin levels, elevating the risk of thrombotic events. These mutations can be detected using PCR-based assays or NGS.[22]

NGS panels targeting multiple thrombophilia-associated genes allow comprehensive screening, particularly in individuals with a strong family history or unexplained recurrent thrombotic events. T-NGS panels can uncover rare or novel mutations, such as those in the *SERPINC1*, *PROC*, and *PROS1* genes, which encode antithrombin, protein C, and protein S, respectively. Such findings can guide clinical management by determining the need for anticoagulation therapy and its duration.[22]

Molecular analysis is also instrumental in assessing acquired thrombophilic conditions. For instance, the presence of the JAK2 V617F mutation indicates an underlying myeloproliferative neoplasm, which often presents with thrombotic complications. In addition, genetic testing can identify conditions with a dual thrombophilic and bleeding risk, such as dysfibrinogenemia, where molecular defects in the fibrinogen gene affect both clot formation and stability.[21]

Advances in molecular diagnostics, including functional assays paired with genetic testing, have improved our understanding of thrombophilia's complex etiology. Molecular findings not only provide insights into thrombosis risk but also inform family screening, genetic counseling, and preventive strategies, such as thromboprophylaxis during high-risk situations such as surgery or pregnancy.

CONGENITAL METHEMOGLOBINEMIAS

Molecular analysis is an essential diagnostic tool for identifying the genetic basis of congenital methemoglobinemia, a rare condition caused by mutations affecting hemoglobin's ability to carry oxygen. This condition can result from mutations in the *CYB5R3* gene encoding cytochrome b5 reductase, or in the globin genes (*HBB* or *HBA1/2*) leading to hemoglobin M (HbM) variants. Molecular testing allows for precise identification of these genetic abnormalities, differentiating between autosomal recessive cytochrome b5 reductase deficiency and dominant HbM disease. NGS techniques are commonly employed to analyze coding exons, splice sites, and relevant regulatory regions of these genes.[23]

Identifying mutations provides insights into the pathophysiology of the disorder—whether it stems from

enzyme deficiency leading to impaired reduction of methemoglobin or from structurally abnormal hemoglobin with reduced electron transfer capacity. For example, mutations in the *CYB5R3* gene can result in Type 1 methemoglobinemia, limited to red blood cells, or Type 2, which involves multiple tissues and presents with severe neurological manifestations. In cases of HbM disease, single-point mutations in the globin genes result in amino acid substitutions that stabilize the ferric (Fe^{3+}) state of heme.[24]

Over 78 mutations have been identified in the CYB5R3 gene, most of which are missense mutations. In a study by Warang *et al*., the Ala179Thr mutation was found to be the most prevalent, occurring in 21.7% of cases.[25]

Molecular analysis aids in confirming the diagnosis, enabling accurate genetic counseling and prenatal testing for families. Additionally, it helps avoid misdiagnosis with acquired forms of methemoglobinemia and provides a foundation for developing targeted therapeutic interventions.

PRIMARY IMMUNODEFICIENCY (PID) AND LYMPHOCYTE FUNCTION DISORDERS

NGS has elucidated the genetic basis of PIDs and congenital disorders of lymphocyte function. Mutations in genes such as *BTK* (X-linked agammaglobulinemia), *IL2RG* (SCID), and *FOXP3* (IPEX syndrome) are now routinely identified, offering insights into pathophysiology and guiding targeted therapies.[26]

CONCLUSION

The integration of NGS technologies into diagnostic practice allows the simultaneous analysis of multiple genes in a single assay at a similar cost to testing a few genes by Sanger sequencing. T-NGS panels, including the protein-coding regions and conserved splice sites of the known genes involved in isolated or syndromic CN, have been developed for the diagnosis of CN. The design of these panels can easily and regularly be updated by integrating the genes most recently reported in literature.

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