JHWCR JOURNAL OF HEALTH, WELLNESS AND COMMUNITY RESEARCH 3007-0570

https://doi.org/10.61919/hrn69k76

Review Article

Regulatory Role of FoxP3 and Immune Dysregulation in β-Thalassemia Major in South Asia: A Systematic Review

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"Cite this Article" Received: 21 April 2026; **Accepted**: 10 May 2026; **Published**: 21 May 2026

Author Contributions: Concept: AA; Design: SAB; Data Collection: MZ; Analysis: HN; Drafting: HI. **Ethical Approval**: New Campus, University of Punjab, Lahore, Pakistan. **Informed Consent**: Written informed consent was obtained from all participants; **Conflict of Interest**: The authors declare no conflict of interest. **Funding**: No external funding; **Data Availability**: Available from the corresponding author on reasonable request; **Acknowledgments**: N/A.

ABSTRACT

Beta-thalassemia major (BTM) continues to be one of the most common inherited hemoglobinopathies in "thalassemia belt" of hemoglobinopathies. In Pakistan, the burden of BTM is comparatively high due to consanguineous marriages and high carrier frequency. Frequent blood transfusion therapy has greatly increased the survival rate of patients but iron overload and long-term transfusion therapy cause persistent immunological dysregulation. According to new research, immunological homeostasis and immune tolerance are primarily regulated by the *FoxP3* (*Forkhead Box P3*) transcriptional factor, and failure of T-regulatory cells (Tregs) may have a significant impact on hemoglobinopathies. This review emphasizes on *FoxP3*-associated genetic polymorphisms such as rs3761548 and rs2232367, which further highlights the current understanding of the molecular and immunological pathways responsible for immune dysfunction in BTM patients. The article provides recent research supporting the validity of the "Multi-Hit Theory" of thalassemic immune failure. Treg inflammatory progression and instability are caused by a combination of chronic antigenic stimulation from frequent transfusions, iron-mediated oxidative damage and inherited genetic susceptibility. Additionally, the present review study emphasizes how clinically relevant outcomes such alloimmunization, leukocyte apoptosis, cytokine imbalance, vascular damage and increased cardiovascular failure are linked to *FoxP3* dysregulation. Exonic and promoter domain of *FoxP3* polymorphisms are highlighted as biological markers that can detect genetically susceptible patients who show acute immune stress. This review also covers the clinical significance of Tetra-Primer Amplification Refractory Mutation System PCR (T-ARMS PCR) as a cost-effective and successful genotyping method ideal for healthcare systems with limited resources. All of the evidence points to the increasing necessity for precision medicine methods based on genetics in the treatment of thalassemia patients. Early detection of immune-related genotypes may enhance chelation therapy, reduce long-term immunological and other pathological problems in susceptible groups and improve transfusion techniques. Keywords: Beta-Thalassemia Major, *FoxP3*, Immune Dysregulation; Polymorphism; Tetra ARMS PCR.

INTRODUCTION

The most important genetically determined diseases are Beta Thalassemia Major (BTM) which is in those areas recognized as the thalassemia belt. This belt covers the Mediterranean basin region to the Middle East region and South Asia region and bears a disproportionate burden of the disease (Weatherall, 2010). In Pakistan, the epidemiological footprint of BTM is there as it has a carrier frequency estimated to be between 5% and 7% (Ansari et al., 2012). The widespread consanguineous marriages and each union's persistence led to a very high increased frequency of homozygous state, resulting in the prevention of around 5,000-9,000 new BTM cases occurring every year across the country as shown in Table-1 (Ahmed et al., 2002). Beta-thalassemia major is a heterogeneous inherited hemoglobinopathy that requires chronic blood transfusions throughout the disease course (Galanello & Origa, 2010). Other researchers found a secondary immunological failure with chronic inflammation and autoantibody production as an increasing life expectancy due to the standardized transfusion protocols proceeded over the years, began to notice something went wrong (Gluba-Brzózka et al., 2021).

Transfusions not only prolong survival but they also induce immune dysregulation leading to autoimmunity, chronic inflammatory diseases and increased risk of infection (Gharagozloo et al., 2013). This review article provides an in-depth narrative study approach to assess the increasing significance of immunological dysregulation and *FoxP3* gene polymorphisms specifically in South Asian populations suffering from β-thalassemia major (BTM). The data was summarized and examined using the web databases such as Google Scholar, ScienceDirect, pertinent scientific literature, PubMed and Scopus published between 1995 and 2026. The study includes different research studies, clinical investigations, epidemiological reports, review articles, immunogenetic studies pertaining to *FoxP3*-associated immune modulation, molecular studies related to Beta-thalassemia major. The main focus of the study is to learn the T-regulatory cell biology, iron metabolism, transfusion-induced immunological dysfunction, oxidative stress pathways, *FoxP3*-associated single nucleotide polymorphisms (SNPs), and epigenetic regulation. BTM is so common in the "thalassemia belt," that's why the research priority regions are South Asian and Middle Eastern populations.

1.1 Pathophysiology and Clinical Impacts

The graphical overview as shown in Figure-1, shows how geography, genetics and clinical pathology all come together to make the study of β-Thalassemia Major in Pakistan and even the larger "Thalassemia Belt" so complicated. It starts by making a map of the areas in the South Asia, Middle East, and Mediterranean where the disease is most common. Pakistan is a major area where thousands of new cases are reported every year because of the increasing percentage of carriers (1). The disease's clinical progression goes from ineffective erythropoiesis and the resulting iron overload to severe immune dysregulation, which damages vital organs like the liver, spleen, and heart throughout the body as shown in Figure-1.

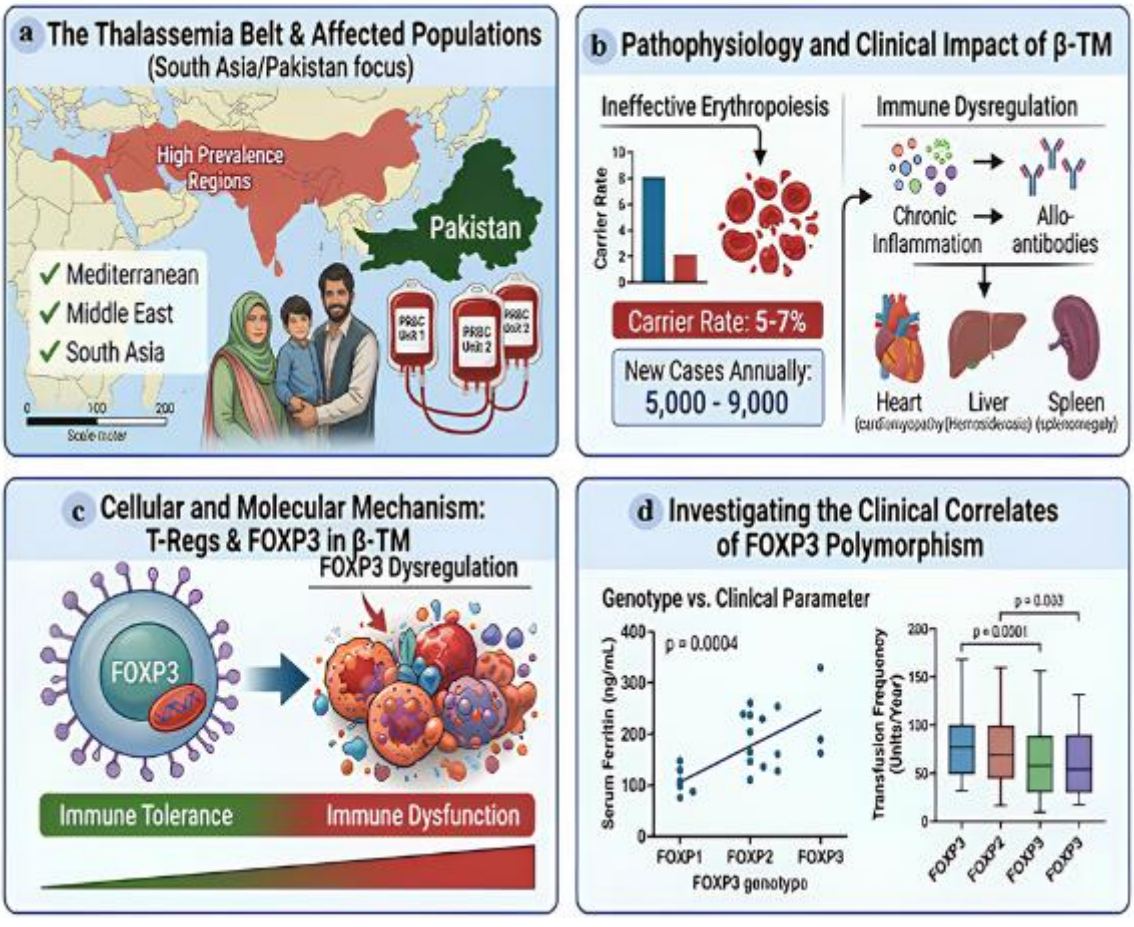

Figure 1: Pathophysiology and Clinical Impact of BTM in South Asian Population

a) Pathophysiology Rationale of β-Thalassemia Major in Pakistan. b) Pathophysiology and the Clinical Impact of BTM on Heart, Liver or spleen. c) Genetic Context of Beta-Thalassemia Major regarding *FoxP3* Immune dysfunction. d) The Clinical correlation of *FoxP3* Polymorphism such as Serum Ferritin Level and Transfusion Frequency (1).

Table 1: Epidemiological Profile of BTM in Pakistan

Parameter	Clinical Value / Statistic
Carrier Frequency	5% – 7% of the general population
Annual New Cases	5,000 – 9,000 cases per year
Primary Risk Factor	High rates of consanguineous marriages
Standard Care	Chronic blood transfusion & iron chelation

1.2 Transfusion-Induced Immune Dysregulation in BTM patients

From a clinical perspective BTM is marked as quantitative deficiency and complete lack of β-globin chain, resulting in the accumulation of unpaired Alpha-chains. These extra Alpha-chains build up in erythroid precursors, which damages the membrane and kills the cells quickly this is called ineffective erythropoiesis. In Pakistan, BTM children survival depends on a lifelong regimen of frequent transfusions of blood and strict therapy of iron chelation. However, some life-saving intervention adds another level of difficulty such as long-term immune stimulation and systemic iron toxicity (2). The function of chronic blood transfusions as a life-sustaining treatment eventually leads to oxidative stress and iron overload. These processes together cause immune dysregulation, which is when T-cell responses change and inflammatory mediators rise (3). Accumulating evidence indicates that *FoxP3* and T regulatory cells (Tregs) play an important role in immune tolerance maintenance (4). The immune homeostasis may be disrupted due to altered expression of Tregs in patients with BTM, and there is need to explore this relationship at molecular level as shown in Figure-2.

β-Thalassemia Major is a monogenic hereditary disorder resulting from β globin gene (HBB) mutations which causes absolute reduction and absence of β globin chain production. This interferes with the production of hemoglobin and makes erythropoiesis ineffective, which leads to severe anemia and a need for regular blood transfusions from a young age. Chronic transfusion, iron chelation and immune dysregulation are observed in such patients which emphasize the regulatory role of *FoxP3*. It leads to post-transfusion microchimerism and alloimmunization. (4). According to the most recent immunological research, thalassemia patients have a complicated immune profile that includes enhanced *CD8+* cytotoxic responses, decreased *CD4+* T cell numbers, dysregulated cytokine production (such as *TNF-α* and *IL-6*), and worsened inflammatory medical conditions as shown in Figure-2 (5).

1.3 The Regulatory Role Immune Tolerance in FoxP3 gene

The investigation is still unclear exactly which regulatory pathways govern immunological homeostasis in BTM. The study of the *FoxP3* transcriptional factor marked a revolutionary breakthrough in our understanding of immune system tolerance. *FoxP3* is consistently identified in the literature as the primary regulator of *CD4+CD25+* Treg cells (6). The primary "brake" on the immune system response is *FoxP3*. In patients who have received numerous transfusions, it prevents T-cells from attacking donor antigens. IPEX syndrome (Polyendocrinopathy, Immune dysregulation, and X-linked Enteropathy), which is best illustrated by mutations characterized by loss of function that cause deadly early-onset autoimmunity, demonstrates the crucial role of *FoxP3*. *RUNX1* and *FoxP3* must cooperate for Tregs to function efficiently (7). Immunosuppression and lymphocyte apoptosis are known outcomes of disturbing this system as shown in Figure-2.

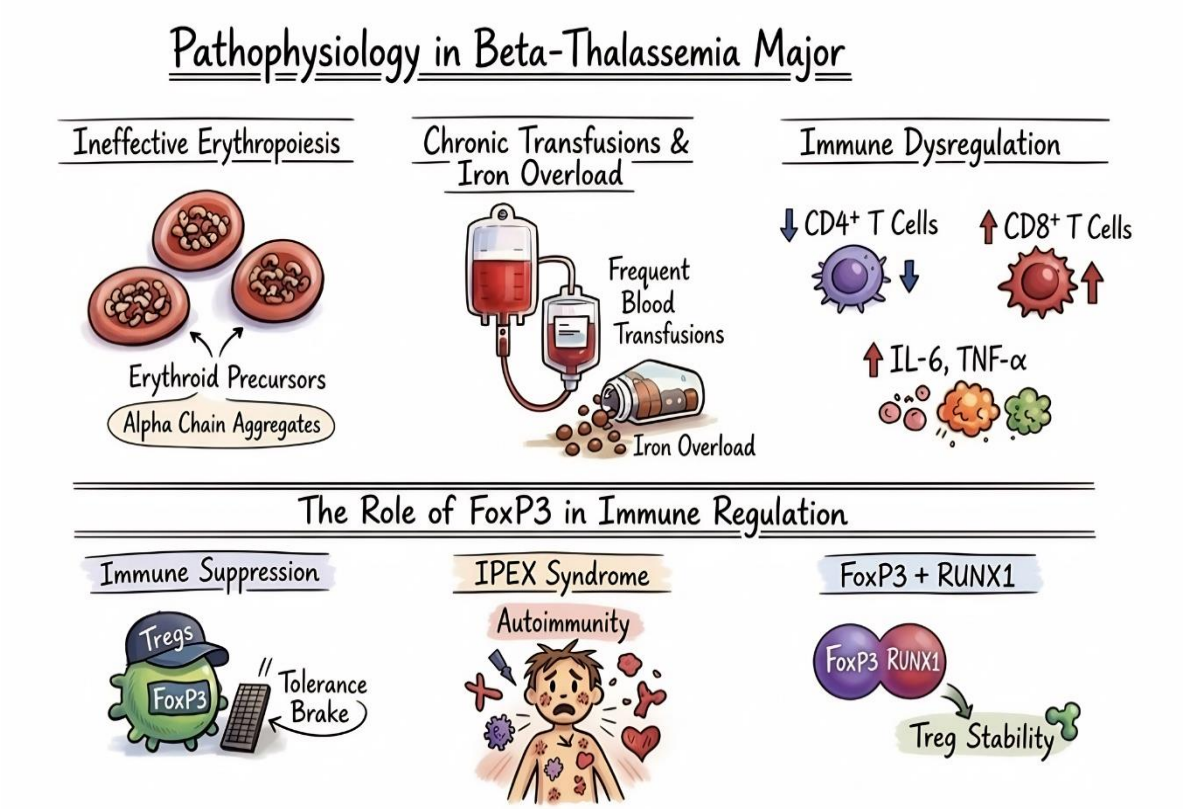

Figure 2: The Role of FoxP3 in Immune Regulation

Various studies showed that the CD4+/CD8+ T-cell ratios are not balanced and the production of cytokine (*IL-6, TNF-α*) is much higher as shown in Table-2. All these affects the autoimmune system which gives strong response. *FoxP3* appears to be a key regulator of T-regulatory cells (Tregs) which shows changes in how T cells expression may lead to problems with immune tolerance and long-term inflammation (4). The role of *FoxP3* function is important in the principle transcriptional factors in preserving immune homeostasis (8).

T regulatory cells that express *FOXP3* act like "tolerance brake" by stopping the immune system from becoming too active. The studies also showed autoimmune diseases like IPEX syndrome are most common due to lack of *FoxP3* protein expression level as shown in Table-2 (9). *RUNX1* has important part in stopping lymphocyte apoptosis, also keeping the T regulatory cells stability. It directly affected by the expression level in *FoxP3* (10). Change in the expression of gene cause immunological dysfunction in most of the patients suffering with β-thalassemia major disorder (11).

According to recent research, people with thalassemia have a variety of immunological disorders. These include altered *CD4+/CD8+* T-cell ratios, increased autoimmune reactions, and altered cytokine secretion patterns (12). These kinds of abnormalities can lead to chronic inflammation and damage the immune system of the body. However, the basic molecular regulators that could explain these irregularities have not received enough attention.

The primary regulatory gene for Tregs, a crucial immunological subgroup that maintains peripheral tolerance and controls inflammation is FoxP3, which is located on chromosome Xp11.23 (13). Autoimmune disorder like IPEX syndrome and other systemic lupus erythematosus disorder have been associated with low FoxP3 expression (14). Examining FoxP3 gene expression in BTM patients may help to clarify the relationship between immunological dysfunction and long-term transfusions (15).

Table 2: Comparative Immunological Profiles

Immunological Marker	Normal Range / Status	BTM Patient Status
***CD4+* T Cells**	Balanced/Homeostatic	Significantly Decreased
***CD8+* Cytotoxic Cells**	Balanced/Homeostatic	Enhanced Response
***CD4+/CD8+* Ratio**	Standard	Inverted Ratio
Cytokines (*TNF-alpha, IL-6*)	Baseline	Pathologically Elevated
Treg Stability	Robust Tolerance	"IPEX-like" Dysfunction

1.4 Alloimmunization in BTM Patients

The potential role of *FoxP3* expression levels can also be used as a biomarker for therapeutic target and immune imbalance. The comparative study of expression level in the patients to healthy controls helps the researchers to manage the problems associated with thalassemia (16).

The principle defect in BTM is hematological; however, the clinical progression of the disease is increasingly characterized by immunological factors (17). Post-transfusion microchimerism is the name for the process by which regular blood transfusions send donor red blood cell (RBC) antigens and donor-derived leukocytes into the recipient's immune system (14). This constant antigenic load keeps the immune system in a state of severe activation as shown in Figure-3.

Alloimmunization is the most important effect of this activation which is when the patient makes antibodies against the antigens in the donor's blood group. In Pakistan, "alloantibody responders" are a big problem in hospitals because it gets harder and harder to find compatible blood, which means that transfusions take longer and people get sicker (12). Also, the immune system has to work in a "toxic" environment where serum ferritin levels are high (18). Advance biochemical studies and researched have illustrated that this excess iron is a strong pro-oxidant that causes leukocyte apoptosis and drives the unbalanced T-cell subsets that are signs of thalassemic immune failure as shown in Figure-3 (19).

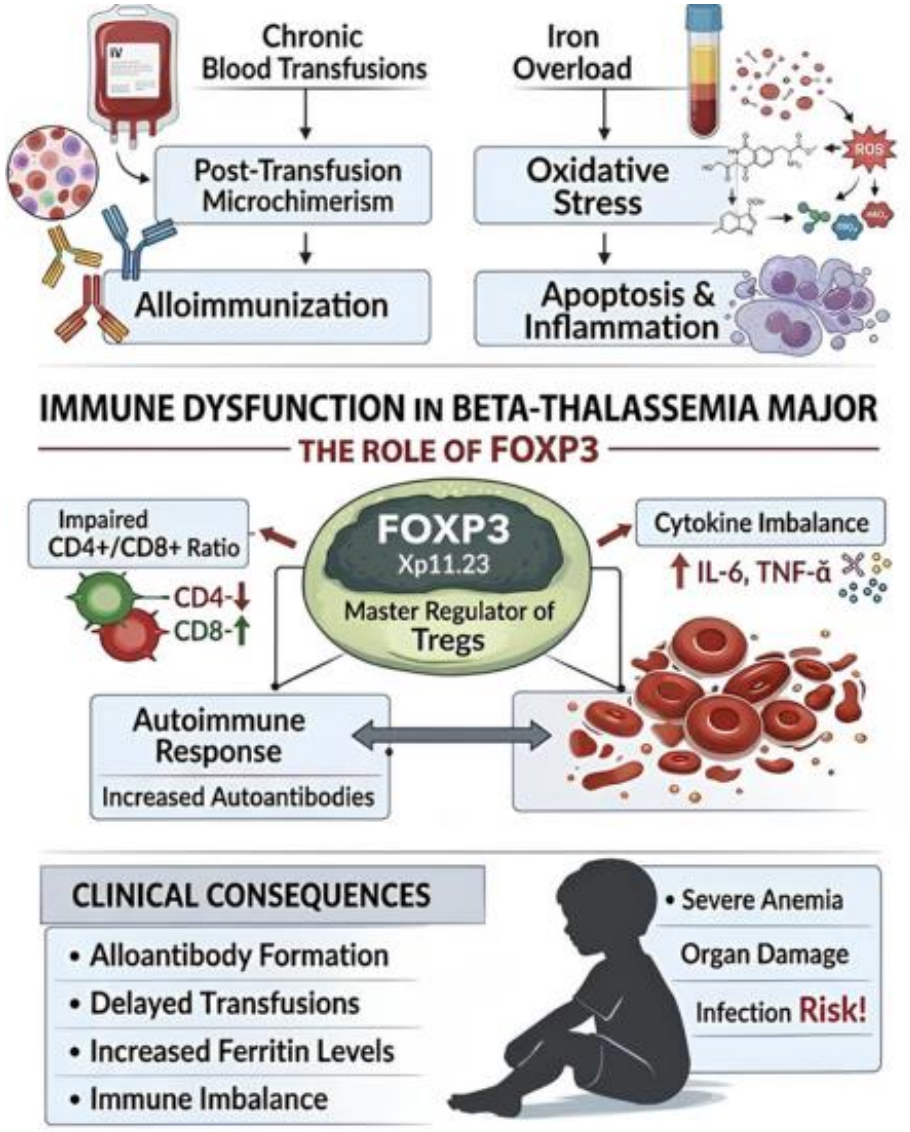

Figure-3: Role of FoxP3 gene (master regulator and Immune Dysfunction in β-thalassemia major patients)

The *FoxP3* transcription factor is at the center of the body's ability to handle this long-term inflammatory stress. *FoxP3* is the main controller of peripheral tolerance and it is mostly found in *CD4+CD25+*Tregs. It acts as the main "brake" on the immune system by stopping the activation of autoreactive and allo-reactive T-cells (13). The value of *FoxP3* is exemplified by IPEX syndrome, characterized as total loss of function by mutations that result in severe, early-onset autoimmunity. Thalassemic patients often have immune dysregulation. Patients usually don't show the full IPEX syndrome phenotype but the condition is "IPEX-like" (20). This study represents the genetic variations caused particularly by the SNP rs3761548 present in the *FoxP3* promoter region. It functions as a molecular rheostat. This polymorphism controls the *FoxP3* protein expression levels. The expression of gene sets the "tolerance threshold" for the patient under stress condition which is triggered by the multiple transfusion of blood as shown in Figure-4. The rs2232367 SNP is an exonic synonymous mutation (c543C>T) that is the specific target. Research shows that synonymous SNPs can change the translational rhythm by altering the mRNA folding which change the translation kinetics. The unchanged amino acid sequence (p.Ser181=) can make proteins less available (21). Tetra-Primer ARM-PCR has been used more and more in regional studies in Palestine, Egypt, and Iraq to quickly find *FoxP3* SNPs because it is cheap and useful for building healthcare systems.

1.5 Multi-Hit Theory of Immune Dysfunction

Contemporary literature endorses a "Multi-Hit" theory for Beta-thalassemia complications including chronic antigenic load, iron oxidative stress and genetic predisposition. Microchimerism after a transfusion and donor RBC antigens get the immune system ready for alloimmunization (22).

High levels of ferritin and Growth Differentiation Factor-15 (GDF-15) in the blood cause the body to generate the Reactive Oxygen Species (ROS) which can deform the DNA present in mitochondria (mtDNA). Leukocyte apoptosis results due to the existence of risk alleles such as rs2232367-A diminishes the threshold for Treg failure (23). The advancement of dysregulation of the immune system is propelled by a series of adverse events. The Hit 1 cause alloimmunization persistent antigenic exposure from transfusions, Hit 2 result in mitochondrial impairment and iron accumulation, and Hit 3 inherited risk alleles diminish *FoxP3* expression (23) as shown in Table-3.

Table 3: Multi-Hit Theory of Immunological Failure

Hit Category	Mechanism of Action	Clinical Outcome
Hit 1: Antigenic Load	Chronic donor RBC antigen exposure	Alloimmunization & microchimerism
Hit 2: Iron Stress	Ferritin/GDF-15 surge; hepcidin suppression	ROS production & mtDNA damage
Hit 3: Genetics	FoxP3 promoter and exonic polymorphisms	Reduced Treg "Tolerance Threshold"

Multiple elements come together to cause a severe, IPEX-like immunological phenotype and a crucial decline in Treg function (24). The *FoxP3* gene structure (chromosomal position Xp11.23) and the regulatory SNPs that establish the tolerance threshold (25) as shown in the Figure-4.

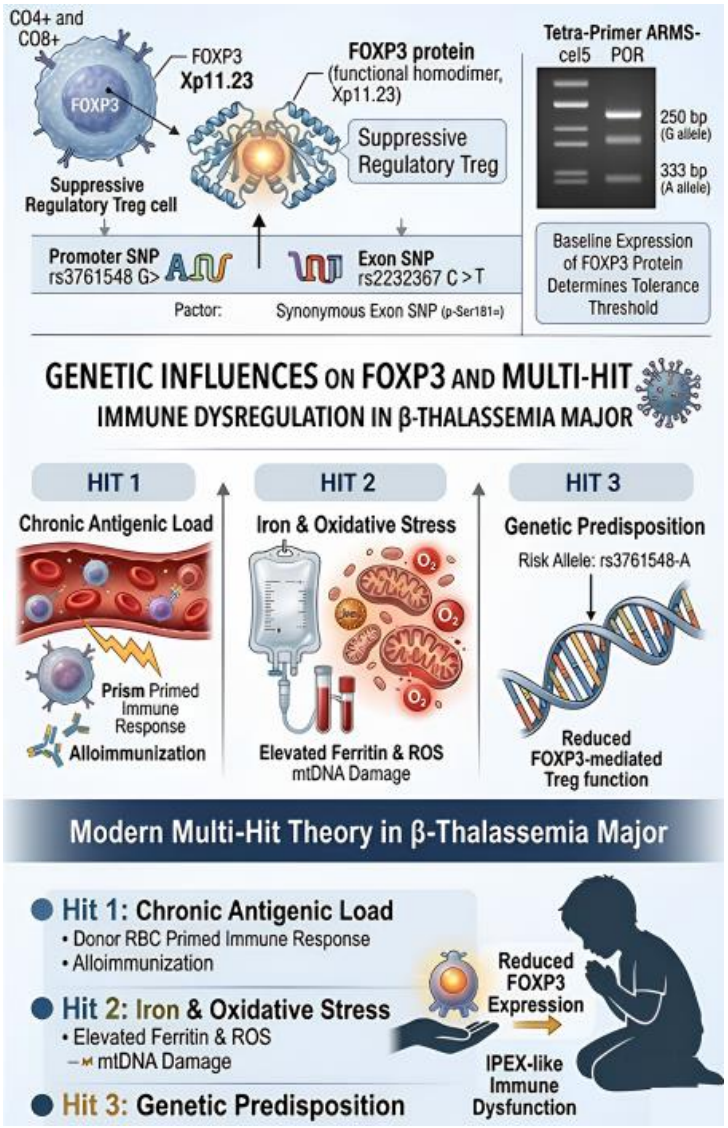

Figure 4: Multi-Hit Model of FoxP3-Mediated Immune Dysfunction in β-Thalassemia Major Patients and its Genetic Influence

1.6 Erythroid-Iron Axis and Epigenetic Silencing

Immune failure in patients with BTM is closely related to the erythroid-iron axis and is not a singular occurrence. Growth Differentiation Factor-15 (GDF-15) and Erythroferrone (ERFE) are the two main erythroid factors that are secreted in large quantities when erythropoiesis is ineffective (26). Master regulator of Iron absorption, hepcidin, is strongly suppressed by these hormones (27). A systemic oxidative stress is brought on by the ensuing increase in non-transferrin-bound iron (NTBI). This oxidative stress encourages the DNA methylation of the *FOXP3* gene promoter, including transcriptome analysis utilizing RNA-Seq (24). This epigenetic silencing becomes more severe in patients with the rs3761548 A-allele, resulting in a significant reduction in T-cell regulatory function. The expression of fatigue markers such *PD-1, LAG-3*, and *CTLA-4* as well as a breakdown in the *RUNX1/FoxP3* axis required for lymphocyte survival, are correlated with this impairment (28, 29). Thalassemic patients' folic acid shortage is directly associated with mtDNA damage and compromised immune gene epigenetic regulation (30). In patients with the AA genotype, these metabolic markers contribute to oxidative dysregulation, which puts additional strain on the already vulnerable Treg population (31).

1.7 The Immuno-Cardiac Axis as Systemic Consequences

There are systemic effects that go beyond the blood when the FoxP3-mediated regulatory system fails. Inflammatory diseases and atopy are more likely to occur when the regulatory safety net is compromised (14, 32). More importantly, the American Heart Association (AHA) has noted that iron-

induced cardiovascular decompensation and a particular thalassemic vascular condition are caused by the chronic inflammatory milieu (32, 33). *CD163* and *TWEAK* (TNF-like weak inducer of apoptosis) are two proteomic markers that have been identified as markers of this persistent macrophage and stress cardiac (34, 35). The rs3761548 SNP is examined in this review article as the primary genetic factor causing failure to multiple organs as shown in Figure-5. In order to create a predictive model that connects this particular genetic variant to the biochemical symptoms (GDF-15, Ferritin) and clinical symptoms of the disease are shown in Figure-5 (36).

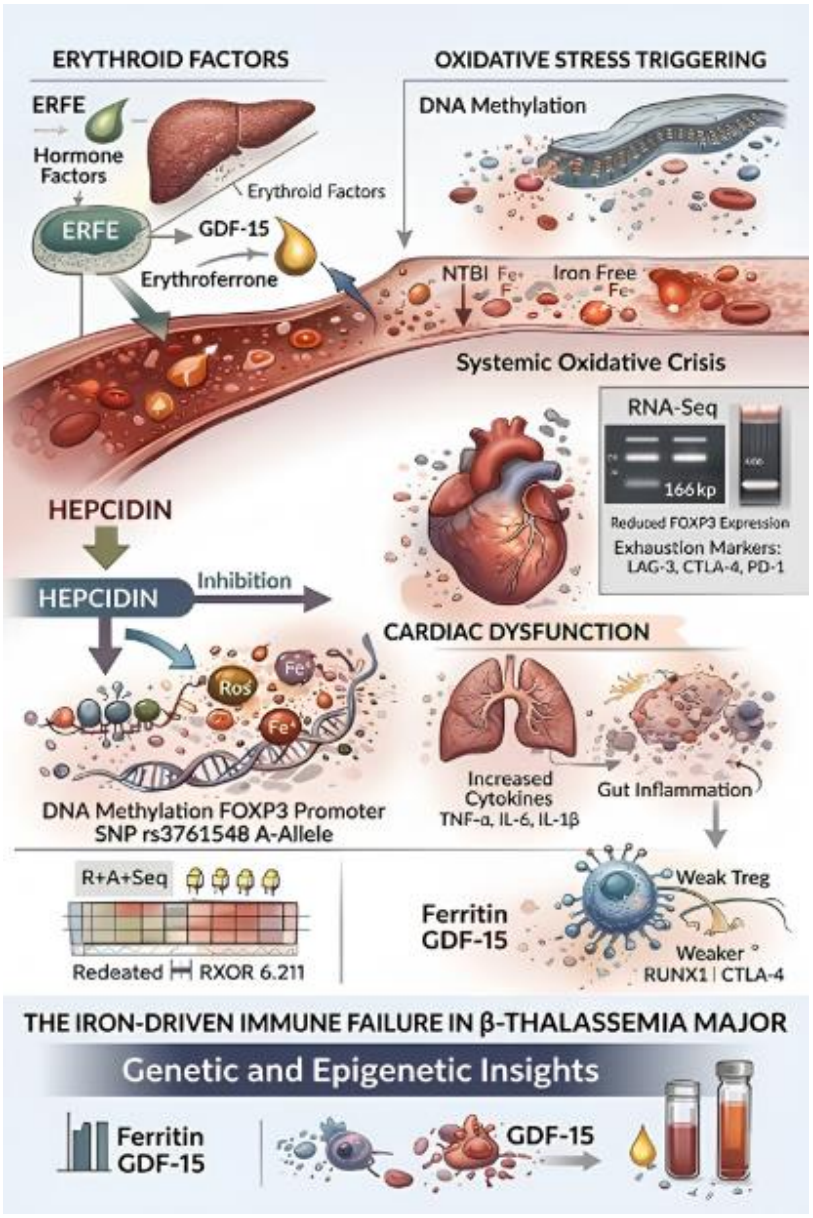

Figure-5: Mechanistic model of the iron-driven immune failure in β-thalassemia major.

Figure-5 represents the schema illustrates how ineffective erythropoiesis and iron overload trigger systemic and molecular-level dysfunction: Erythroid and Oxidative Triggering: Enhanced production of erythroferrone (ERFE) and other erythroid factors (including the diagnostic biomarker GDF-15) by the bone marrow suppresses hepcidin (37, 38).

This suppression causes unlimited dietary and transfusional iron absorption in the context of inadequate red blood cell formation, which results in severe iron overload and high Ferritin (39). Reactive oxygen species are increased when Non-Transferrin-Bound Iron (NTBI) causes a systemic oxidative crisis (40). At the *FoxP3* promoter domain, elevated levels of iron and ROS cause epigenetic DNA methylation, which severely suppresses *FoxP3* expression as shown by RNA-seq and other experiments in

Wawrusiewicz-Kurylonek et al study (41). Furthermore, this pathway is interfered with by the particular SNP rs3761548 (A-allele), which may reduce *RUNX1* binding and destabilize Treg function. Immune failure resulting from these types of assaults can be identified by "exhausted" T cells (upregulation of *CTLA-4*, *PD-1*) and a poor Treg phenotype (42). A self-reinforcing cycle of immunological instability and severe inflammation is created when iron-mediated gut inflammation and multi-organ (cardiac) injury are combined (43).

Although promoter variants have received a lot of attention in the past, current scholarly immunogenomic research emphasizes the important role of exonic polymorphisms. The X chromosomes *FoxP3* gene's exon region has the rs2232367 SNP (44, 45). The transition comprises c.543C>T substitution at the molecular level. The variants classified as a synonymous mutation (p.Ser181=) are becoming more widely known for their capacity to affect splicing efficiency mRNA stability, and, translational kinetics which in turn modifies the final protein pool accessible for T-regulatory cell activity (46). Minor alterations in *FoxP3* expression can have significant clinical consequences. The enormous "antigenic load" resulting from several transfusions in the Pakistani population which has become a major problem. The discovery of the G and A alleles at the rs2232367 locus offers a genetic marker for patient of Beta-Thalassemia stratification according to their innate immune response (47, 48).

1.8 TETRA-ARM PCR for Stratified Care

The practice of Tetra-Primer Amplification Refractory Mutation System PCR (T-ARM PCR) implies to do high-throughput, economical genotyping in an environment with limited resources (23) as shown in Table-4. PCR is especially useful since it eliminates the need for costly post-PCR procedures like RFLP or sequencing by detecting both alleles in a single reaction tube as shown in Table-4 (49-51).

Table 4: Diagnostic and Clinical Utility of TETRA-ARM PCR

Feature	Tetra-Primer ARMS-PCR Strategy	Clinical Benefit
Cost-Efficiency	Eliminates post-PCR RFLP or sequencing	Ideal for low-resource settings
Speed	Detects both alleles in a single reaction	High-throughput patient screening
Precision	Distinct bands for G (250bp) and A (333bp)	Clear "Responder" stratification

The rs2232367 genotype with the systemic markers associated with β-Thalassemia. Pediatric β-Thalassemia Major (BTM) patients in Pakistan show significant clinical variability despite standardized chelation methods and blood transfusion as shown in Figure-6 (52). Despite controlled serum ferritin levels, a significant subset of these patients, known as "high-responders," undergo accelerated cardiovascular vasculopathy and fast alloimmunization as shown in Table-5 (53). The reason why some children experience immunological fatigue and early leukocyte apoptosis while others maintain relative stability cannot be explained by conventional clinical markers as shown in Figure-7 (54). The local population-specific genetic modifiers of tolerance to immunological factors are mostly unknown. In particular, nothing is known about the function of exonic polymorphisms in the *FoxP3* gene, like rs2232367, in relation to South Asian thalassemic populations (55). Clinicians cannot tailor care without determining these genetic risk variables, which results in a standardized strategy that might not be adequate for children who are genetically sensitive as shown in Figure-8 (56).

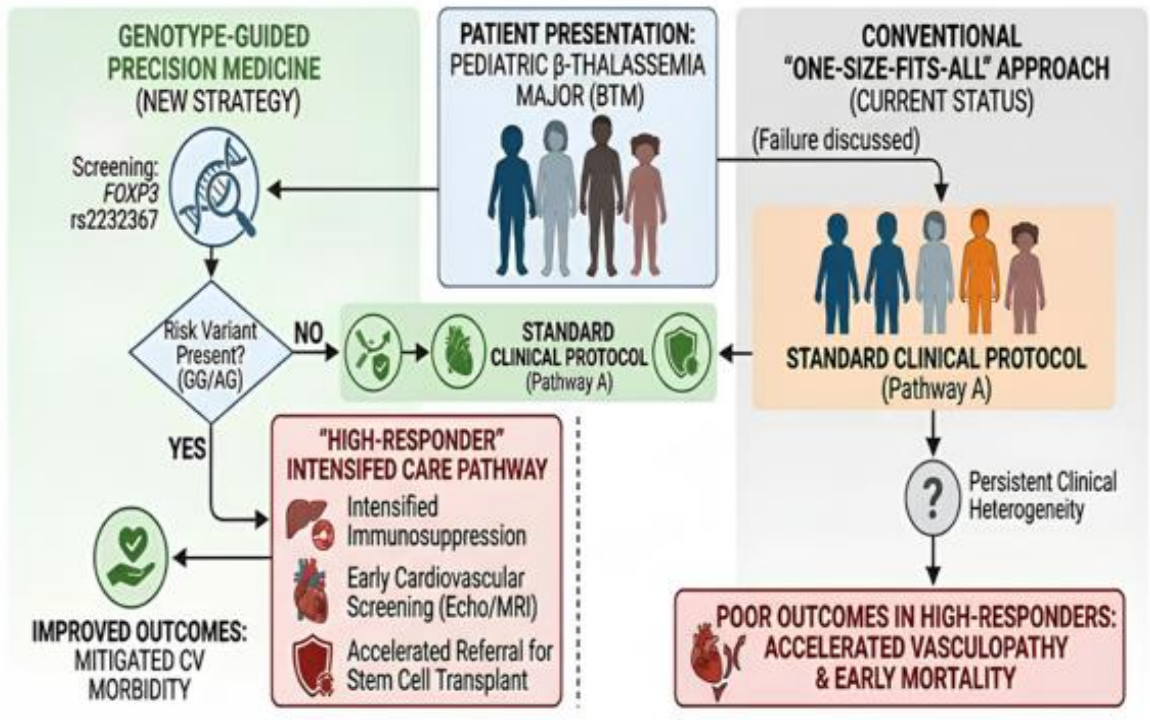

Figure-6: Proposed stratified Care Model for Pediatric BTM in Pakistan

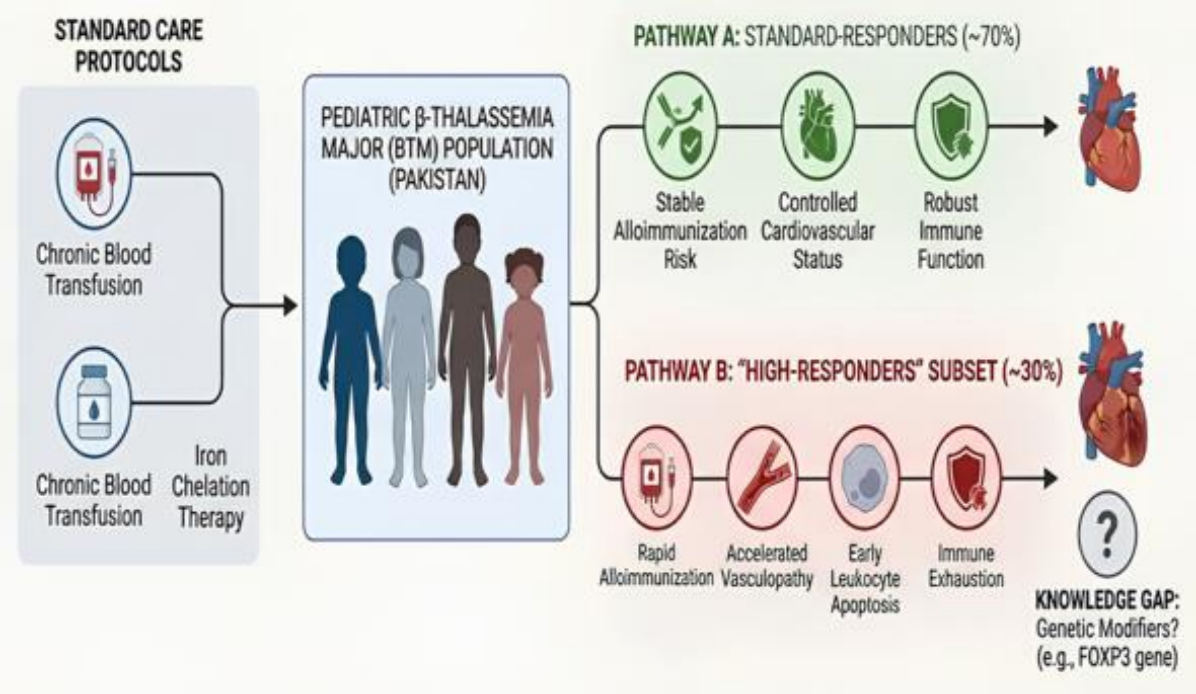

Figure-7: Conceptual Model of Clinical Heterogeneity in Pediatric BTM Patients

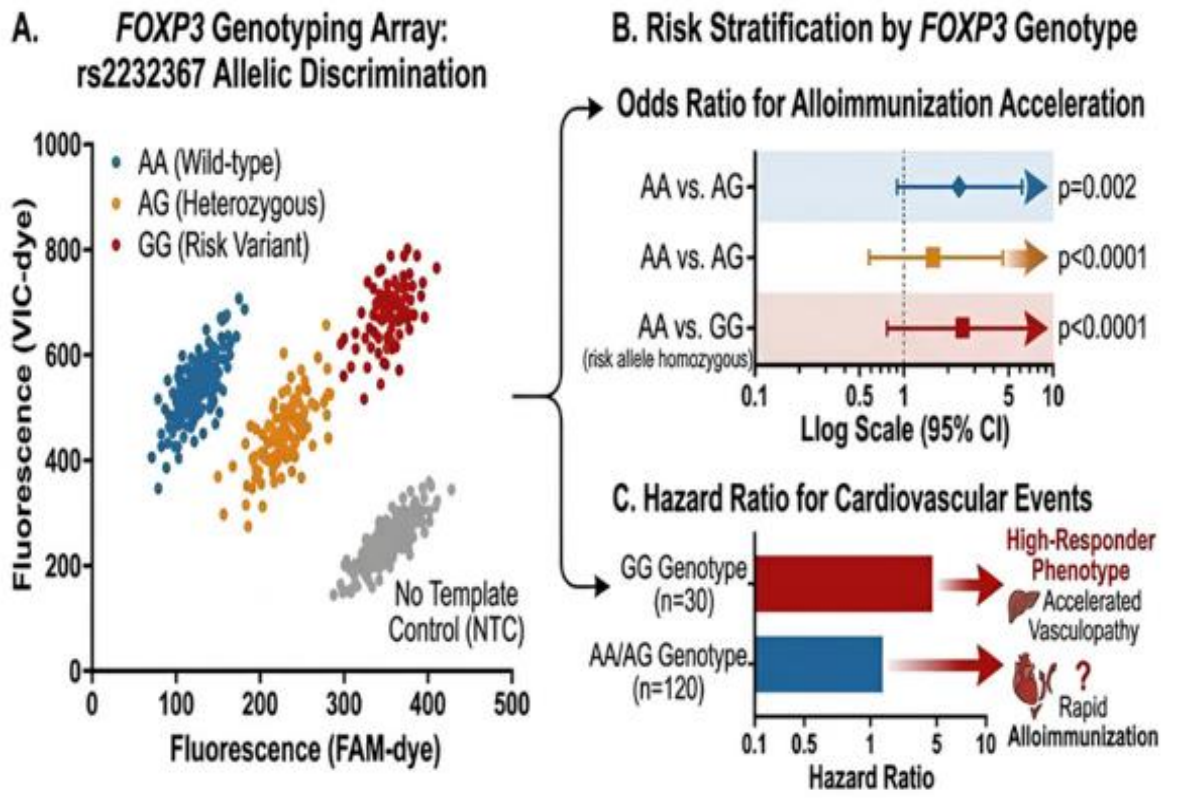

Figure-8: Association of FoxP3 Genotypes with Clinical Heterogeneity

A) *FoxP3* SNP rs2232367 polymorphism showing the adverse clinical effect. B) odd ratio for alloimmunization acceleration and significance level demonstrated by the Wild type AA, Heterozygous AG and Mutant GG variants on risk. C). The Hazard ratio shown by the mutant allele for Cardiovascular failure in BTM patients comparative to other wild type and heterozygous alleles as shown in Table-5.

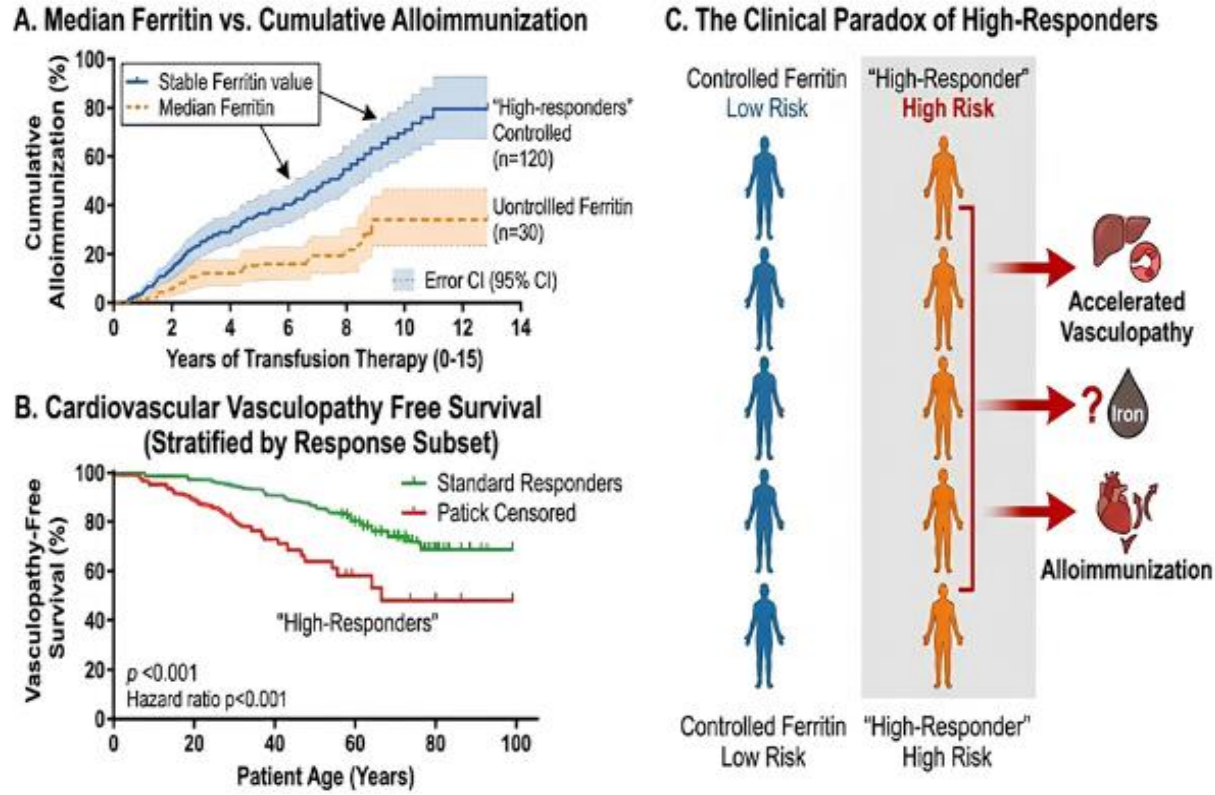

Figure-9: Retrospective Analysis of Clinical Outcomes vs. Ferritin Level represents the high responders in correlation with the frequent transfusion of blood

A) The Median Ferritin level vs. Cumulative alloimmunization which remains high in High responders. B) Cardiovascular vasculopathy survival rate for High responders is low due to significant risk ratio. C) The clinical paradox of high- responders illustrate the hazard risk which is more due to their elevated ferritin level which ultimately makes the situation of patients worse as shown in Figure-9.

Table 5: Stratified Care Model: "Standard" vs. "High-Responders"

Patient Subset	Genetic Risk Variant	Recommended Clinical Pathway
Standard-Responder	GG/AG Genotype	Standard chelation & transfusion protocol
High-Responder	AA Genotype (Risk Allele)	Intensified chelation & immunosuppression
Monitoring Priority	Routine	Accelerated Cardiovascular Echo/MRI

For the Pakistani healthcare system, where the prevalence of BTM is still an important issue for the public's health, this research has significant socioeconomic and clinical importance (57). This study establishes a scientific basis for the application of stratified medicine in hematological care by pinpointing a particular genetic limitation within the *FoxP3* gene (23, 58, 59). This technique has cheap operating costs so it is a perfect option for widespread screening in resource-constrained environments like those found in the interior of Pakistan. In the end, these discoveries can direct preventive measures, such customized HbF augmentation and more aggressive chelation therapy for genetically susceptible "responders," lowering the long-term risk of immunological and cardiac problems. Focusing on the rs2232367 (c.543C>T) synonymous mutation is justified by the growing knowledge that exonic single nucleotide polymorphisms (SNPs). It can significantly change the "translational rhythm" of the *FoxP3* protein (60). These mutations can affect the patterns of protein folding and mRNA stability, despite their similarities. According to this study, a more severe immune system exhaustion phenotype is linked to the A allele, which is identified by the IF-G and IR-A primers. It is also suggested that the level of oxidative stress and damage in mtDNA seen in thalassemic leukocytes will be directly correlated with genotypic changes at this locus, potentially compromising the epigenetic stability of the *FOXP3* gene sequence (61).

This review article investigates the Immuno-Cardiac Axis, in which cardiac decompensation is accelerated by constant systemic inflammation, indicated by biomarkers like *CD163* and TWEAK particularly reported in specific thalassemic vasculopathy, pediatric cohorts, and vascular stiffness result from *FoxP3* inability to adequately control this systemic inflammation (62). The enlargement of the compartments of erythroid in the bone marrow is directly correlated to the degree of inefficient erythropoiesis (26). ERFE and GDF-15 become important hormonal messengers throughout this process (27). Hepcidin which is the master Iron regulator usually suppressed due to the pathologically high levels of ERFE and GDF-15. It results in excessive macrophage iron release and uncontrolled intestinal iron absorption (63). Serum ferritin, which continues to be the predominant indicator for global iron load in Pakistani thalassemic children, is pathologically elevated as a result of this endocrine dysregulation (64). The Fenton reaction is facilitated by the ensuing iron overload, which produces extremely reactive radicals known as hydroxyl (65). A vicious cycle of immunological dysfunction and oxidative stress is created when these radicals directly damage mitochondrial DNA and alter the epigenetic landscape (66).

The immune system experiences early senescence and functional depletion in the setting of BTM (67). Unbalanced T-cell subsets, particularly an inversion of the *CD4/CD8* ratio and a marked decline in the absolute count of *CD4+* and *CD25+* T- regulatory cells (Tregs) (3, 68). Both T and B lymphocytes undergo apoptotic pathways in response to oxidative stress and chronic inflammation; this process is primarily controlled by the stable functioning of the *RUNX1/FOXP3* regulatory pathway (9, 69). Additionally, checkpoint markers like *LAG-3* and *CTLA-4* have been analyzed to be upregulated in thalassemic Peripheral Blood Mononuclear Cells (PBMCs) by transcriptome studies (RNA-Seq) (69, 70). his increase indicates that the regulatory "safety net" has failed, making patients susceptible to alloimmunization and

infections. Finding the rs2232367 A-allele may enable medical professionals to classify individuals as "high-responders" who are more likely to acquire antibodies against donor blood antigens, making long-term transfusion management even more challenging (29, 71).

The study demonstrate the structure of Beta Thalassemia Major (BTM), the immune system is not merely a passive observer of hematological failure but an active participant in the disease's clinical trajectory (44). The active role of immune system shows in the clinical course of Beta Thalassemia Major (BTM) rather than being a passive witness of hematological failure (4). In order to prevent the formation of alloantibodies after repeated blood transfusions, the immunological synapse—the point where T-cells engage with antigen-presenting cells (APCs)—needs a high level of regulatory control (72). The main "molecular rheostat" in this synapse is *FoxP3*, the master transcription factor found on chromosome Xp11.23. Peripheral tolerance depends on the stability of *FoxP3*-specific T- regulatory cells (Tregs) (73). The immune system is primed for a state of chronic activation in BTM patients because to the continuous infusion of donor donor-derived leukocytes and red blood cell (RBC) antigens, a process known as post-transfusion microchimerism (74). The "tolerance brake" is released when the *FoxP3* gene is unable to coordinate the proper immunosuppressive response, which causes alloantibodies to develop quickly (75). This is especially important in the clinical situation in Pakistan, where "alloantibody responders" have a very hard time getting matching blood, which directly raises rates of mortality as well as morbidity (71, 76).

Treg plasticity is significantly influenced by the *FoxP3* gene epigenetic environment in addition to its main genetic sequence. BTM is represented by rising level of ROS which also include a "systemic oxidative crisis" caused by NTBI (64, 77). According to recent studies, this oxidative stress encourages the FoxP3 promoter's DNA methylation, hence suppressing its expression (78). The Fenton reaction is triggered by elevated serum ferritin, which functions as a pro-oxidant in the iron-driven epigenetic cycle. Mitochondrial Fragility is the process produces hydroxyl radicals that directly harm mitochondrial DNA (mtDNA), further straining the immune system's ability to regulate (65). The *RUNX1* Interaction is the possible reduction in the binding affinity of *RUNX1*. It is a protein required for maintaining the *FoxP3* complex, exacerbates this epigenetic silencing in patients with the rs2232367 A-allele (9). Because genetic susceptibility and epigenetic alteration work together, *FOXP3* levels may be an important predictive biomarker for immunological dysregulation (79).

Pakistan has a distinct sociocultural context that contributes to the epidemiological burden of BTM (57, 80);. (81). The high frequency of consanguineous marriages, which raises the possibility of homozygous genetic states for both the primary β-globin mutations and secondary genetic modifications, such the *FOXP3* rs2232367 polymorphism, is a major contributor to this prevalence (82, 83). For individuals who are genetically inclined to "high-responder" status, the "standard" strategy to chelation and transfusion frequently proves inadequate in settings with limited resources (84). The Pakistani healthcare system can move toward a differentiated care paradigm by including immunogenomic screening, especially by employing the affordable T-ARMS-PCR technique (85). For children with the A-allele are most at risk for accelerated immunological exhaustion and vasculopathy, this would enable the prioritizing of early cardiovascular screening and aggressive chelation (86, 87).

The immune system frequently mimics the functional depletion experienced in persistent infections or cancer as the BTM clinical trajectory advances (88). Exhaustion markers such as *LAG-3* and *CTLA-4* are upregulated in this "premature senescence": These indicators show that the regulatory "safety net" has failed, leaving the patient open to opportunistic infections (89). *CD4/CD8* Ratio Inversion: A drop in absolute Treg numbers is often associated with *CD4/CD8* Ratio Inversion, which is a characteristic of thalassemic immune failure. The *RUNX1/FOXP3* axis is regulated by chronic inflammation, and both B and T cells undergo apoptosis when this axis is disrupted (9). We open the door for future medical precision techniques, like adoptive Treg immunotherapy or targeted gene modulation, to restore

equilibrium in these susceptible juvenile cohorts by comprehending the genetic bottleneck caused by the rs2232367 SNP (90, 91).

CONCLUSION

A complex "Multi-Hit" structure of immunological failure, in which chronic antigenic load, iron-driven oxidative stress and intersect with genetic susceptibility is increasingly defining the clinical development of beta-Thalassemia Major (BTM) in Pakistan. The *FoxP3* transcription factor, which functions as a fundamental molecular rheostat and is essential to this dysregulation, is severely disrupted by some single nucleotide polymorphisms, such as rs3761548 and rs2232367. These genetic variations provide a baseline "tolerance threshold" that distinguishes a susceptible "high-responder" subset among patients who are particularly susceptible to rapid alloimmunization and accelerated vasculopathy. Genotype-guided precision medicine must be incorporated into the national healthcare system in order to overcome the deficiencies of the existing "one-size-fits-all" treatment procedures. In areas with limited resources, the use of inexpensive diagnostic technologies like Tetra-Primer ARMS-PCR provides an appropriate approach for early risk classification. For the purpose of reduction in the persistent burden of immunological or cardiac problems in the Pakistani BTM population, doctors might prioritize increased chelation and personalized transfusion techniques by identifying genetically susceptible pediatric population.

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