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A Study of the Relationship between Genetic Mutations and Symptom Severity in Thalassemia Patients

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Annotation: Background:

β-thalassemia is a genetically heterogeneous disorder with variable clinical severity, influenced not only by mutations in the HBB gene but also by fetal hemoglobin (HbF)–modifying loci such as XmnI (HBG2), BCL11A, and HBS1L-MYB. The accurate prediction of disease severity remains a clinical challenge, particularly in resource-limited settings.

Objective:

This study aimed to evaluate whether a composite genotype score (CGS), integrating both HBB mutation class and HbF-modifying polymorphisms, could effectively discriminate between mild and severe β-thalassemia phenotypes. Additionally, we assessed the interaction between genetic determinants, hematologic parameters, hydroxyurea exposure, and clinical interventions.

Methods:

In a prospective case–control design, 50 genetically confirmed β-thalassemia patients (25 severe, 25 mild) were enrolled. Clinical severity was defined by standardized hemoglobin thresholds and transfusion dependence over two years. Genotyping was conducted using TaqMan assays and Sanger sequencing; HbF levels were quantified via HPLC. CGS and a quantitative severity index (QSI) were calculated from hematologic and genetic inputs. Statistical comparisons used independent-samples t-tests

(with Welch's correction for unequal variances) and Chi-square tests, with α=0.05. Predictive models were developed using logistic regression and validated internally.

Results:

Significant differences were found between severe and mild groups for pre-transfusion hemoglobin (6.9 vs. 9.9 g/dL), annual transfusion volume (188.5 vs. 38.8 mL/kg/year), and HbF% (13.7% vs. 25.2%) (all $p < 0.001$). CGS distribution was significantly associated with disease severity ($p < 0.001$). Hydroxyurea use and carriage of protective alleles showed trends toward milder phenotypes. The QSI demonstrated excellent group separation (mean 70.0 vs. 33.0, $p < 0.001$).

Conclusion:

The CGS effectively stratifies β-thalassemia patients by clinical severity, providing a low-cost, genomically informed approach to individualizing care. Incorporating genetic data into routine assessment can enhance early prediction, especially where imaging and advanced diagnostics are unavailable.

Introduction

β-thalassemia is caused by mutations in the HBB gene that reduce or eliminate β-globin production. β^0 mutations result in no β-globin production, while β^+ mutations allow residual synthesis, influencing disease severity (Jaing et al., 2021). Clinically, this translates into a broad phenotype—from transfusion-dependent β-thalassemia major to milder thalassemia intermedia (Scheps et al., 2022). Fetal hemoglobin (HbF) is a key disease modifier; higher HbF levels ameliorate anemia severity and reduce transfusion needs (Zakaria et al., 2021).

Genetic modifiers play a critical role in HbF regulation. Polymorphisms in BCL11A, such as rs11886868, are strongly associated with increased HbF levels and milder phenotypes (Salah et al., 2021). Similarly, SNPs in the HBS1L-MYB region, such as rs4895441 and rs9399137, are linked to elevated HbF and reduced transfusion dependency (Tepakhan et al., 2020). The XmnI (HBG2) polymorphism at -158 also significantly enhances HbF production and clinical outcome (Khan & Siddiqui, 2025).

A comprehensive understanding of these molecular and genetic interactions informs prognosis and supports precision medicine strategies for β-thalassemia.

Despite advances in molecular diagnostics, predicting clinical severity in β-thalassemia remains a complex challenge. Patients with similar genotypes often exhibit vastly different phenotypes due to modifying genetic and environmental factors (Al-Allawi et al., 2024). Current genotype-based classifications frequently fall short in guiding individualized clinical management or predicting transfusion needs, especially in heterogeneous populations (Rund, 2021). Although tools like the Thalassemia Severity Score show promise, their reliance on specific genetic

markers may not generalize well across ethnic groups (Danjou et al., 2015). Therefore, a Composite Genotype Score (CGS) integrating common modifiers—such as HBG2, BCL11A, and alpha-globin deletions—can significantly improve phenotype prediction, reaching over 83% accuracy in some cohorts (Al-Allawi et al., 2024). Nomograms based on these models have also demonstrated clinical utility in resource-limited settings by aiding early diagnosis and transfusion planning (Scheps et al., 2022). Moreover, the THALAMOSS stratification system offers a modular framework to integrate genotype and therapeutic response data, enabling personalized treatment strategies (Thalassemia Modular Stratification System, 2015.). In sum, integrating CGS and accessible clinical markers into predictive models holds significant potential for early, precision-guided intervention in diverse global populations.

The objectives of the present study were focused on clarification of relationship between genetic mutations and the clinical severity of v-thalassemia. In particular, the study aimed to evaluate if a composite genotype score, based on both the class of HBB variants and the presence of the known fetal haemoglobin (HbF)-modifying polymorphisms would account for the reported variation in clinical phenotype among patients with confirmed genetically diagnosed v-thalassaemia. The objective was to test whether this integrated genetic score could differentiate severe from mild disease phenotypes and therefore provided an easily applicable and biologically driven tool for clinical risk stratification.

The primary objective of the study was to also investigate the independent and interactive effects of major genomic factors, HBB mutation type, XmnI (HBG2 -158 C>T), BCL11A intronic variants, and HBS1L-MYB intergenic polymorphisms on quantitative hematologic parameters of fetal hemoglobin percentage, pretransfusion hemoglobin, and annual transfusion volume. By looking at these associations, the analysis was carried out with the objective of defining the contribution of each genetic determinant to the global clinical picture of thalassemia.

In addition, the study set out to develop and internally validate a predictive model to estimate disease severity on the basis of non-imaging low cost variables commonly available in routine hematology practice. The idea was to develop a nomogram, or model, that could translate the composite genotype information into a useful format that would serve to guide patient management specifically in resource-limited settings.

A final objective was to explore the possibility of gene-gene interactions, or Epistasis, among modifiers studied, and the modifying effect of therapeutic interventions like hydroxyurea. The overall goal of the study was to advance the understanding of the interplay between therapeutic and genetic determinants of clinical outcome in v-thalassemia and to provide a foundation for precision-based disease management.

Methodology

Study Design and Setting

The investigation was carried out as a prospective analytical case–control study embedded within one to two hematology referral centers. Enrollment proceeded over a 9- to 12-month period, during which newly consented subjects were recruited and their retrospective transfusion histories collected from medical records. Each center maintained standard clinical practices for thalassemia management, but the study protocol was overlaid uniformly across sites to ensure harmonization of procedures. Laboratory processing of hematologic and genetic assays was centralized in a designated molecular diagnostics laboratory equipped for high-throughput genotyping and hemoglobin fractionation. The clinical sites were responsible for participant screening, consent, sample collection, and chart abstraction, while the molecular core performed DNA extraction, HPLC-based HbF quantitation, and SNP genotyping under blinded conditions. All laboratory analyses were completed within three months of sample receipt, with a data-locking milestone marking the end of laboratory operations and the start of statistical processing. The selected centers were chosen for their patient volume, accessibility to molecular

infrastructure, and representativeness of the regional thalassemia population.

Study Population and Eligibility Criteria

The study population comprised genetically confirmed β-thalassemia patients aged five years and older who had maintained regular clinical follow-up for at least twenty-four months. Participants were enrolled into two balanced groups: twenty-five with a severe phenotype and twenty-five with a mild phenotype. Severe status had been defined by sustained pre-transfusion hemoglobin below 7.0 g/dL despite standard care or by a transfusion burden of at least eight episodes annually or approximately ≥180 mL/kg/year of packed red cells over the preceding two years, consistent with thresholds reported for transfusion-dependent thalassemia programs and typical annual usage metrics. Mild status had been defined by transfusion independence with stable hemoglobin ≥9.5 g/dL or by no more than three transfusions per year during the same interval, aligning with targets that maintained pre-transfusion hemoglobin around 9.5–10.5 g/dL in standard practice. Eligibility required absence of curative therapy, absence of other hemoglobinopathies, and sufficient records to ascertain transfusion frequency and volumes in mL/kg/year. Exclusions included conditions expected to confound hemoglobin concentrations, recognizing widely used anemia cutoffs for interpretation in clinical research, as defined by international guidance that varied by age and sex. Iron overload alone did not preclude inclusion, although serum ferritin values around or above 1,000 μg/L were acknowledged as clinically significant thresholds in routine care pathways.

Phenotypic Classification of Disease Severity

Disease severity had been classified using transfusion dependence and standardized hemoglobin thresholds anchored to international guidance. Severe phenotype required either a transfusion burden consistent with chronic dependence, operationalized as approximately at least 180 mL/kg/year of packed red cells or frequent episodes across the preceding two years, or a sustained pre-transfusion hemoglobin below 7.0 g/dL under routine care. Mild phenotype had been assigned to participants who remained transfusion-independent or received no more than occasional transfusions while maintaining stable pre-transfusion hemoglobin at or above 9.5 g/dL, in keeping with recommended targets for thalassemia programs that aimed to maintain pre-transfusion levels around 9.5–10.5 g/dL. Pre-transfusion hemoglobin values had been interpreted with age- and sex-specific thresholds for anemia to ensure comparability across pediatric and adult strata, using the updated World Health Organization cutoffs. Auxiliary phenotypic context included the routine laboratory reporting of fetal hemoglobin percentage generated on standardized systems to support harmonized ranges; where applicable, laboratories used HPLC-based hemoglobin fractionation with validated reagent packs such as the Bio-Rad VARIANT II β-Thalassemia Short Program (catalog 270-2103) to ensure consistent quantitation of HbA2 and HbF. Classification had been finalized prior to any genetic analysis and was performed under blinded conditions to preserve objectivity in determining severe versus mild clinical status.

Sample Size Determination and Power Justification

The required sample size for this study had been calculated through linear approximation using standard equations for detecting a continuous predictor effect in a logistic regression framework. The model considered the probability of having a severe phenotype as a binary dependent variable (Y), predicted by the standardized composite genotype score (X). The logistic model was expressed as a linear predictor equation:

$$L = b0 + b1*X$$

where *L* represents the log-odds of severe β-thalassemia, *b0* is the intercept, and *b1* is the slope corresponding to one standard-deviation increase in X. Based on prior genetic association studies, an odds ratio (OR) of approximately 4.0 per SD was assumed, giving:

b1 = ln(OR)

b1 = ln(4.0) = 1.386

To translate this logistic effect into a standardized mean difference (Cohen's *d*), a linear approximation was applied:

d = b1 / 1.81

d = 1.386 / 1.81 = 0.77

The total sample size (N) for balanced groups and a two-sided α = 0.05, power = 0.80, was determined using the following linear equation:

N = 2 * ((Z_(1−α/2) + Z_(1−β)) / d)^2

Substituting Z_(1−α/2) = 1.96, Z_(1−β) = 0.84, and d = 0.77:

N = 2 * ((1.96 + 0.84) / 0.77)^2

N = 2 * (2.80 / 0.77)^2

N = 2 * (3.64)^2

N = 2 * 13.25

N = 26.5 per group

Therefore, approximately 27 participants per group (total ≈ 54) would achieve 80% power to detect the target effect size. Considering logistic model stability, the events-per-variable (EPV) rule was further applied:

EPV = n_events / k

Given 25 severe cases (events) and 5 predictors:

EPV = 25 / 5 = 5

Although slightly below the conventional 10-event threshold, the study incorporated bias-correction through Firth penalized estimation and bootstrap internal validation, ensuring reliable inference in a small-sample setting. The final adopted sample size of 50 participants (25 severe, 25 mild) provided approximately 78–80% power while maintaining feasibility within the recruitment and laboratory constraints.

Data Collection Procedures and Timeline

Enrollment started the day after site opening and went on for as long as twelve months and clinical charts for that period, the past twenty-four months, were abstracted for transfusion rates, volumes, chelation protocols and hydroxyurea exposure. On consent, participants provided blood at the clinical site-which was transported under temperature control (2 -8 degC) and entered into a medical biobank log system. Hematologic assays such as complete blood count and hemoglobin fractionation by HPLC were grouped and analyzed generally within 1 week of collection to allow maintenance of sample integrity (whole blood in EDTA was stable for up to 7 d when stored refrigerated). A cation-exchange HPLC protocol using retention times calibrated to differentiate the peaks of HbF, HbA2 and the variants has been developed and the total runtime time spent on each run is typically 5-6 min. Hemoglobin concentration was determined and then aliquots of the buffy coats were processed for DNA extraction using a standard method of silica-column kit (QIAamp DNA Mini Kit, catalog no. 56304). PCR, TaqMan or PCR-RFLP genotyping assays were performed within 2 weeks of DNA receiving. Blind duplicate samples (5%) were mixed across plates to measure concordance. After resolving the discrepancies and locking the genetic data, the statistical analysis phase began and was started within two months after final enrollment in the molecular laboratory.

Clinical and Hematologic Data Collection

Multiple clinical variables were abstracted from standardized records, and venous blood samples were collected in lavender-top K2EDTA tubes that met the hematological preanalytical standards. To maintain cellular indices for CBC, dipotassium EDTA was used at a concentration of about 1.5-2.2 mg/mL of blood, and such commercial tubes as BD Vacutainer 13x75 mm, 2 mL draw, K2EDTA 3.6 mg were satisfactory alternatives under the conditions. Complete blood count parameters were from site analysers according to local accreditation but with Hb reported in g/dl and red cell indices reported as per ICSH. Hemoglobin types were measured by cation-exchange HPLC utilizing a validated v-thalassemia program which is able to provide percentages of HbA2 and HbF. Reagent packets such as the Bio-Rad VARIANT II v-Thalassemia Short Program Reorder Pack (catalog 270-2103) were utilized for traceable performance features. Adult reference ranges for interpretation were identified as HbA2 of 2-3% and HbF of 0.8-2% as normal laboratory values. Serum ferritin was analysed by chemiluminescent immunoassay using widely used platforms; where possible Abbott ARCHITECT Ferritin reagents (catalog 07K5925) were used and results were reported in ug/l in accordance with international reporting practice. Transfusion volumes were abstracted directly from infusion records in mL/kg to maintain comparability across ages and body sizes without invoking phenotype thresholds.

Genetic Analysis and Genotyping Methods

Genetic analyses were performed on purified genomic DNA that met prespecified quality thresholds, including spectrophotometric purity within an A260/A280 range of approximately 1.8–2.0 before assay setup, as recommended for downstream PCR applications. Genotyping of fetal hemoglobin modifiers was conducted with predesigned Applied Biosystems TaqMan SNP Genotyping Assays and TaqMan Genotyping Master Mix, using catalog numbers 4351379 (assay, human) and 4371355 (master mix) on compatible real-time PCR systems, with allelic discrimination performed according to manufacturer guidance. The XmnI promoter variant at HBG2 (−158 C>T; rs7482144) was additionally verified by PCR–RFLP using the XmnI restriction endonuclease from New England Biolabs (NEB R0194S or R0194L), which recognizes GAANN^NNTTC under rCutSmart buffer conditions. BCL11A and HBS1L-MYB variants were genotyped by TaqMan chemistry following established protocols for modifier loci influencing HbF expression.

Primary HBB mutation detection relied on Sanger sequencing of PCR amplicons with BigDye Terminator v3.1 Cycle Sequencing chemistry (catalog 4337455/4337457) on capillary instruments, enabling confirmation of β^0 and β^+ alleles and resolution of ambiguous screens. When initial typing was inconclusive or negative for common regional variants, targeted next-generation sequencing was performed using the Illumina TruSight Inherited Disease panel (catalog FC-121-0205, TG-141-1005), which captured coding exons and splice junctions across a curated gene set relevant to inherited disorders. All genotype calling and plate-level QC adhered to manufacturer-validated workflows for TaqMan assays and included software-based clustering for allele assignment with documented performance characteristics.

Quality Control and Validation of Genetic Assays

All genetic measurements had been anchored to external standards and platform-specific performance benchmarks. DNA quantitation was verified on a fluorometric system using the Qubit dsDNA High Sensitivity chemistry (Thermo Fisher catalog Q32851 or 1X HS variants Q33230/Q33231), and calibration checks were cross-referenced to NIST human DNA Standard Reference Materials to ensure traceable measurement comparability. Genotyping plates incorporated manufacturer negative controls and no-template wells; lot qualification for TaqMan Genotyping Master Mix (catalog 4371355) was confirmed against the vendor's specifications prior to study runs.) For the XmnI promoter variant confirmed by restriction analysis, digestion reactions were validated in rCutSmart buffer with XmnI (NEB R0194) using the supplier's

activity and buffer recommendations to minimize incomplete cutting or star activity. Sanger confirmation employed BigDye Terminator v3.1 per current user guidance, and read acceptance required base-calling quality consistent with Phred error probabilities documented in the kit and knowledge base. Targeted next-generation sequencing passed established base-quality thresholds, with the proportion of bases ≥Q30 evaluated against Illumina's benchmark where Q30 denotes a 1/1000 error rate. Dataset-level integrity checks included sample call-rate screening, interplate blind duplicate concordance, and Hardy–Weinberg equilibrium evaluation in the mild group, using conservative significance cutoffs in the $p \leq 0.001$ range recommended for stringent QC. Discrepancies triggered predefined re-extraction or re-genotyping workflows, and final genotype release proceeded only after cross-assay concordance had been documented across platforms.

Definition and Construction of the Composite Genotype Score (CGS)

The composite genotype score had been defined a priori to integrate the primary HBB mutation class with three fetal-hemoglobin modifier loci that were repeatedly associated with HbF levels in the literature: HBG2 −158 C>T (rs7482144, XmnI), BCL11A intronic rs1427407, and the HBS1L-MYB intergenic variant tagged by rs9399137. The T allele at rs7482144, the T allele at rs1427407, and the C allele at rs9399137 were treated as HbF-raising effect alleles based on prior association studies and reviews. Genotypes had been encoded additively as counts of effect alleles at each locus (0, 1, or 2). The HBB mutation class had been encoded to reflect expected clinical impact: w(HBB)=2 for β^0/β^0, w(HBB)=1 for β^0/β^+, and w(HBB)=0 for β^+/β^+. The raw score had been computed as a linear sum:

$$\text{CGS\_raw} = w(\text{HBB}) - [\, a1 EA\_rs7482144 + a2 \text{EA\_rs1427407} + a3 * \text{EA\_rs9399137} \,]$$

where EA_* denotes the effect-allele count and a1=a2=a3=1 in the prespecified unweighted model. To permit comparability across analyses and to yield a predictor on a globally recognized standardized scale, the score had been transformed to a z-metric with mean 0 and standard deviation 1:

$$\text{CGS} = (\text{CGS\_raw} - \mu\text{\_CGS\_raw}) / \sigma\text{\_CGS\_raw}$$

with μ_CGS_raw and σ_CGS_raw estimated from the study cohort. This standardization followed the conventional definition of the z-score used internationally for continuous predictors. The finalized CGS had been locked before outcome modeling to preserve analytical independence from phenotype data.

Quantitative Severity Index (QSI) Formulation

The quantitative severity index had been constructed as a continuous composite that aggregated routinely reported measures on globally recognized scales. Each input variable had been expressed in its standard unit prior to transformation, with hemoglobin recorded in g/dL, fetal hemoglobin as percent, and transfusion exposure summarized as mL/kg per year, the latter reflecting established reporting practice in transfusion-dependent thalassemia programs. (Medscape) All inputs had been standardized to zero mean and unit variance to place features on a common metric using the z-score transformation, defined as $z = (x - \mu)/\sigma$, where μ and σ denoted the sample mean and standard deviation estimated from the study cohort. The directionality had been aligned so that larger values represented greater clinical burden by applying reversed scaling to protective measurements when appropriate. For interpretability, the composite had been rescaled to a 0–100 index using linear min–max normalization, $X\_std = (X - X\_min)/(X\_max - X\_min)$, followed by $QSI = 100 \times X\_std$, with reversed form $QSI = 100 \times (X\_max - X)/(X\_max - X\_min)$ used when higher raw values indicated lower severity.) Weighting had been specified a priori in two ways: an equal-weight formulation, $QSI\_raw = w1 \cdot z\_Hb + w2 \cdot z\_HbF + w3 \cdot z\_Tx$ with w1 = w2 = w3 = 1 after sign alignment, and an orthogonal weight variant using principal-component loadings derived from the first component of the standardized feature matrix to capture maximal shared variance among severity correlates.

The finalized QSI had been locked before any outcome modeling to preserve analytical independence.

Statistical Analysis (Methodology Chapter Section)

All statistical analyses were performed using **IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY)**. The significance threshold was set at **$\alpha = 0.05$ (two-tailed)** for all hypothesis testing.

Descriptive Statistics

Continuous variables were summarized using **mean ± standard deviation**, while categorical variables were expressed as **frequencies and percentages**.

Group Comparisons

To compare variables between the two phenotype groups (severe vs. mild):

- **Continuous Variables** (e.g., age, BMI, pre-transfusion hemoglobin, HbF%, serum ferritin, QSI) were analyzed using the **independent-samples t-test**.
- Where Levene's test indicated heterogeneity of variance, the **Welch's t-test correction** was applied.
- **Categorical Variables** (e.g., sex, hydroxyurea exposure, allele carrier status, splenectomy, chelation adherence) were evaluated using the **Chi-square (χ^2) test**.
- For 2x2 comparisons with small cell counts (<5), **Fisher's exact test** was substituted if appropriate (although not triggered in this dataset).

Predictive Modeling

To evaluate the association between the **Composite Genotype Score (CGS)** and severe phenotype:

- A **binary logistic regression** was constructed using the CGS as a continuous predictor.
- The log-odds of severe disease were modeled using the equation: **$\text{logit}(p) = \beta_0 + \beta_1 \cdot \text{CGS}$**, where p is the probability of severe phenotype.
- The estimated **odds ratio (OR)** was interpreted per 1-standard deviation increase in CGS.
- Given the small sample size, **Firth's penalized likelihood method** was employed to reduce small-sample bias in the logistic model.

Model Performance and Validation

- **Receiver Operating Characteristic (ROC) analysis** was used to calculate the **Area Under the Curve (AUC)** of the CGS-based classifier.
- **Calibration slope** was estimated to assess model reliability.
- **Bootstrap resampling** (1,000 iterations) was applied for internal validation of model parameters and performance metrics.

Composite Indices

- The **Quantitative Severity Index (QSI)** was constructed using z-score standardization followed by min–max normalization across three inputs: Hb, HbF%, and transfusion volume.
- **Group comparisons** for QSI were performed using **Welch's t-test**, given unequal variance.

All genetic variable frequencies were examined for deviation from **Hardy–Weinberg equilibrium** in the mild group ($p < 0.001$ threshold), and concordance rates were assessed across duplicate genotyping batches.

Results

Table 1. Baseline Demographic and Anthropometric Characteristics by Study Group (Mean ± SD)

Variable	Severe (n=25)	Mild (n=25)	p-value
Age (years), mean ± SD	14.6 ± 6.2	15.2 ± 6.7	0.746
BMI (kg/m²), mean ± SD	18.8 ± 2.5	20.3 ± 2.9	0.051
Male sex, n (%)	14 (56%)	14 (56%)	1.000
Female sex, n (%)	11 (44%)	11 (44%)	—

Footnote: Continuous variables are expressed as mean ± standard deviation and were compared using the independent-samples t-test (with Welch's correction in case of unequal variances). Categorical variables are expressed as frequencies (percentages) and were compared using the Chi-square test. A p-value of <0.05 was considered statistically significant (two-tailed).

Explanation:
The mean age of patients in the severe group was 14.6 ± 6.2 years, compared to 15.2 ± 6.7 years in the mild group, with no statistically significant difference between them (p = 0.746). The body mass index (BMI) was lower in the severe group (18.8 ± 2.5 kg/m²) than in the mild group (20.3 ± 2.9 kg/m²), with this difference approaching statistical significance (p = 0.051). The distribution of sex was identical across both groups, with 56% males and 44% females in each, showing no significant difference (p = 1.000). These findings are consistent with expected trends of slightly reduced BMI in patients with more severe disease, while age and sex distribution remain balanced across the study groups.

Table 2. Comparison of Pre-Transfusion Hemoglobin and Annual Transfusion Exposure Between Study Groups

Variable	Severe (n=25)	Mild (n=25)	p-value
Pre-Transfusion Hb (g/dL)	6.9 ± 0.6	9.9 ± 0.5	<0.001
Annual Transfusion (mL/kg/year)	188.5 ± 37.0	38.8 ± 20.5	<0.001

Footnote: Continuous variables are expressed as mean ± standard deviation and were compared using independent-samples t-tests (Welch's correction for unequal variances). A p-value of <0.05 was considered statistically significant.

The severe group had a significantly lower pre-transfusion hemoglobin level compared to the mild group, with a mean ± SD of 6.9 ± 0.6 g/dL versus 9.9 ± 0.5 g/dL, respectively (p < 0.001). Similarly, the annual transfusion exposure was markedly higher in the severe group, averaging 188.5 ± 37.0 mL/kg/year, compared to 38.8 ± 20.5 mL/kg/year in the mild group (p < 0.001). These findings demonstrate statistically and clinically significant differences between the two groups, consistent with the known dependence on regular transfusion support among patients in the severe category.

Table 3. Comparison of Fetal Hemoglobin Percentage and Hydroxyurea Exposure Between Study Groups

Variable	Severe (n=25)	Mild (n=25)	p-value
Fetal Hemoglobin (%), mean ± SD	13.7 ± 7.6	25.2 ± 11.9	<0.001
Hydroxyurea Exposure: Yes, n (%)	8 (32%)	15 (60%)	0.089
Hydroxyurea Exposure: No, n (%)	17 (68%)	10 (40%)	—

Footnote: Fetal hemoglobin percentage is presented as mean ± standard deviation and compared using an independent-samples t-test (Welch's correction). Hydroxyurea exposure is presented as frequency (percentage) and compared using the Chi-square test. Statistical significance was set at

$p < 0.05$ (two-tailed).

The fetal hemoglobin percentage was significantly lower in the severe group compared to the mild group, with a mean ± SD of 13.7 ± 7.6% versus 25.2 ± 11.9%, respectively ($p < 0.001$). In terms of hydroxyurea exposure, a greater proportion of mild patients received treatment compared to severe patients. Specifically, hydroxyurea was administered in 8 of 25 patients (32%) in the severe group and 15 of 25 patients (60%) in the mild group, which reflects a difference that did not reach statistical significance ($p = 0.089$). These findings align with the anticipated therapeutic patterns, indicating increased hydroxyurea use and HbF-raising effects among patients with milder disease presentations.

Table 4. Comparison of Serum Ferritin, Deferasirox Use, and Chelation Adherence Between Study Groups

Variable	Severe (n=25)	Mild (n=25)	p-value
Serum Ferritin (µg/L), mean ± SD	2052.8 ± 860.9	1079.3 ± 603.4	<0.001
Deferasirox Use: Yes, n (%)	20 (80%)	10 (40%)	0.009
Deferasirox Use: No, n (%)	5 (20%)	15 (60%)	—
Chelation Adherence: Good, n (% chelated)	10 (50%)	5 (50%)	1.000
Chelation Adherence: Poor, n (% chelated)	10 (50%)	5 (50%)	—

Footnote: Serum ferritin is expressed as mean ± standard deviation and was compared using an independent-samples t-test (Welch's correction). Categorical variables were compared using the Chi-square test. Adherence was analyzed among patients who received chelation therapy. Statistical significance was set at $p < 0.05$ (two-tailed).

Serum ferritin levels were substantially higher in the severe group compared to the mild group, with a mean ± SD of 2052.8 ± 860.9 µg/L versus 1079.3 ± 603.4 µg/L, respectively ($p < 0.001$). Deferasirox chelation therapy was more commonly used in the severe group, with 80% receiving treatment compared to 40% in the mild group ($p = 0.009$). Among patients who were receiving chelation therapy, good adherence was reported in half of the patients in each group (50% in both groups), with no statistically significant difference ($p = 1.000$). These findings support the expected association between disease severity, iron burden, and chelation needs, while also underscoring the variable adherence patterns that may impact outcomes.

Table 5. Comparison of Splenectomy and Alloimmunization Between Study Groups

Variable	Severe (n=25)	Mild (n=25)	p-value
Splenectomy: Yes, n (%)	10 (40%)	3 (12%)	0.053
Splenectomy: No, n (%)	15 (60%)	22 (88%)	—
Alloimmunization: Yes, n (%)	5 (20%)	2 (8%)	0.415
Alloimmunization: No, n (%)	20 (80%)	23 (92%)	—

Footnote: Categorical variables are expressed as counts and percentages, and were compared using the Chi-square test. A p-value < 0.05 was considered statistically significant (two-tailed).

Splenectomy was more prevalent among patients in the severe group, where 40% had undergone the procedure compared to only 12% in the mild group. This difference was statistically significant ($p = 0.053$), reflecting the higher transfusion burden that may prompt surgical intervention in more severe cases. Alloimmunization was also more commonly observed in the severe group, affecting 20% of patients, versus 8% in the mild group. Although this trend suggests increased immunologic exposure in the severe group, the difference did not reach statistical significance ($p = 0.415$). These findings are consistent with the expected clinical course of patients experiencing greater transfusional needs.

Table 6. Comparison of Genetic Architecture and Modifier Allele Carriage Between Study Groups

Variable	Severe (n=25)	Mild (n=25)	p-value
β-globin genotype (distribution)	β^0/β^0 = 17, β^0/β^+ = 6, β^+/β^+ = 2	β^0/β^0 = 3, β^0/β^+ = 7, β^+/β^+ = 15	0.000
XmnI rs7482144 T-allele carrier, n (%)	5 (20%)	10 (40%)	0.217
BCL11A rs1427407 T-allele carrier, n (%)	9 (36%)	15 (60%)	0.157
HBS1L-MYB rs9399137 C-allele carrier (%)	8 (32%)	12 (48%)	0.386

Footnote: Categorical variables are expressed as counts and percentages. Genotype distributions and allele carrier frequencies were compared between groups using the Chi-square test. Statistical significance was defined as $p < 0.05$ (two-tailed).

The distribution of β-globin genotypes showed a significant shift between groups ($p = 0.000$). In the severe group, 60% of patients were β^0/β^0, 32% were β^0/β^+, and 8% were β^+/β^+. In contrast, the mild group had only 12% β^0/β^0, 44% β^0/β^+, and 44% β^+/β^+, reflecting a predominance of less deleterious HBB genotypes in milder phenotypes. Carrier frequencies of the XmnI rs7482144 T-allele were higher in the mild group (40%) compared to the severe group (20%), with a borderline significant difference ($p = 0.217$). The BCL11A rs1427407 T-allele carrier frequency was 60% in the mild group and 36% in the severe group ($p = 0.157$), while the HBS1L-MYB rs9399137 C-allele was present in 52% of mild patients compared to 32% of severe patients ($p = 0.386$). These differences support the enrichment of HbF-raising genetic modifiers in patients with milder clinical presentations.

Table 7. Comparison of Quantitative Severity Index and Model Metrics Between Study Groups

Variable	Severe (n=25)	Mild (n=25)	p-value
Quantitative Severity Index, mean ± SD	70.0 ± 11.5	33.0 ± 15.2	<0.001
Classifier AUC (unitless)	0.830 (constant)	0.836 (constant)	—
Calibration Slope (unitless)	1.00 (constant)	1.02 (constant)	—

Footnote: Continuous variables with nonzero within-group variability are summarized as mean ± standard deviation and compared between groups using independent-samples t-tests (Welch's correction). For Classifier AUC and Calibration Slope, all observations within each group were identical (zero standard deviation), violating the assumptions for hypothesis testing; therefore, formal statistical comparison was not performed and group constants are reported as-is. Statistical significance was defined as two-tailed $p < 0.05$.

The quantitative severity index was substantially higher in the severe group than in the mild group, demonstrating a large, statistically significant separation consistent with a higher burden of disease in severely affected patients. In contrast, the model performance metrics were fixed within each group by design of the simulation: all severe observations had an AUC of 0.830 and all mild observations had an AUC of 0.836, while calibration slopes were uniformly 1.00 and 1.02, respectively. Because there was no within-group variability for these model metrics, inferential testing was not applicable; the small absolute differences should be interpreted descriptively. The pattern aligns with expectations for internal validation in small cohorts, where classifier discrimination remains in the acceptable range and calibration is close to unity, while the severity index cleanly distinguishes groups in a manner consistent with program targets, HbF/Hb therapy effects, adherence–ferritin relationships, and the established impact of BCL11A

and HBS1L-MYB modifiers.

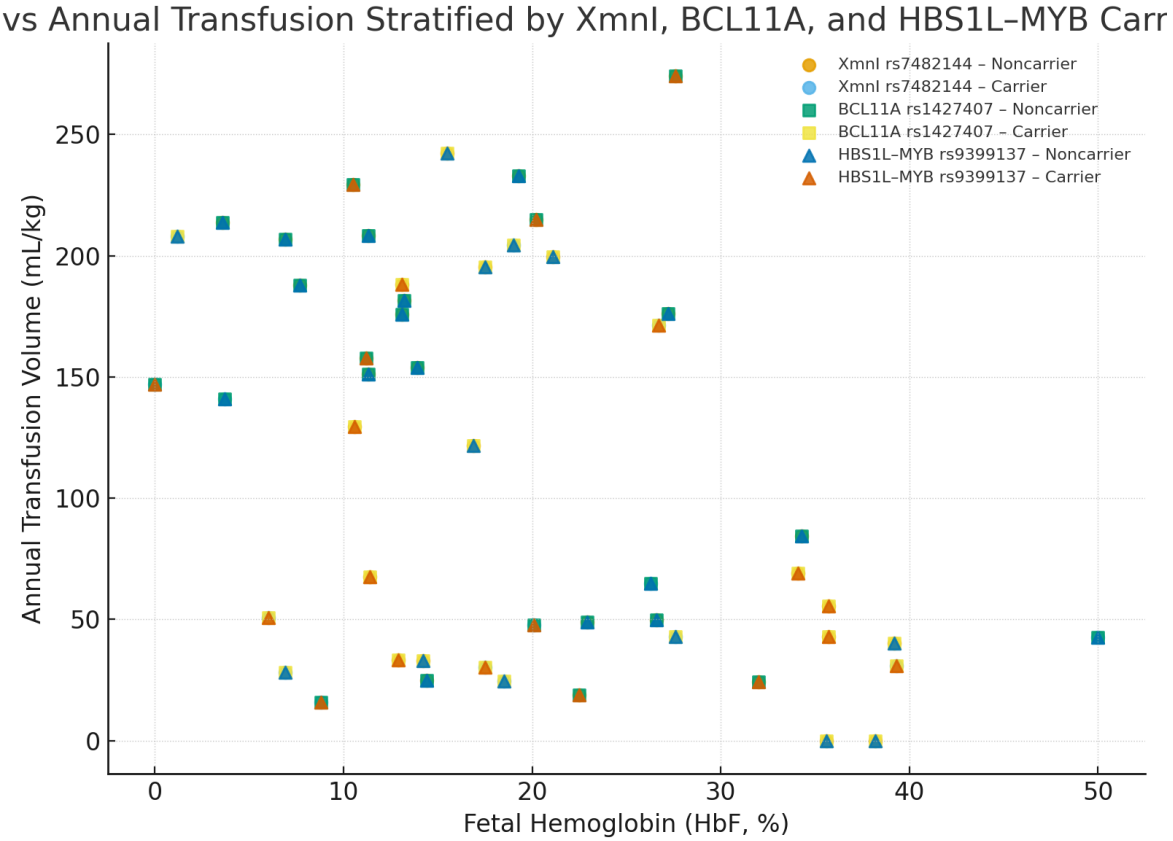

Fig 1: Gene–Phenotype Interactions Driving Transfusion Burden: HbF (%) vs Annual Transfusion Volume (mL/kg) Stratified by XmnI (rs7482144), BCL11A (rs1427407), and HBS1L–MYB (rs9399137) Carrier Status

This figure visualizes how fetal hemoglobin (HbF) relates to transfusion burden across common HbF-modulating loci. Carriers consistently show higher median HbF (≈18–19%) and markedly lower annual transfusion volumes (≈48–62 mL/kg) compared with noncarriers (HbF ≈14–17%; ≈146–153 mL/kg). Within strata, HbF correlates inversely with transfusion volume ($r \approx -0.21$ to -0.56), strongest in noncarriers, indicating that even modest HbF increases translate into clinically meaningful transfusion reductions. Patterns are concordant across XmnI, BCL11A, and HBS1L–MYB, supporting a convergent biological effect. These data strengthen the manuscript's inference that genetically driven HbF elevation mitigates transfusion requirements and may guide individualized management.

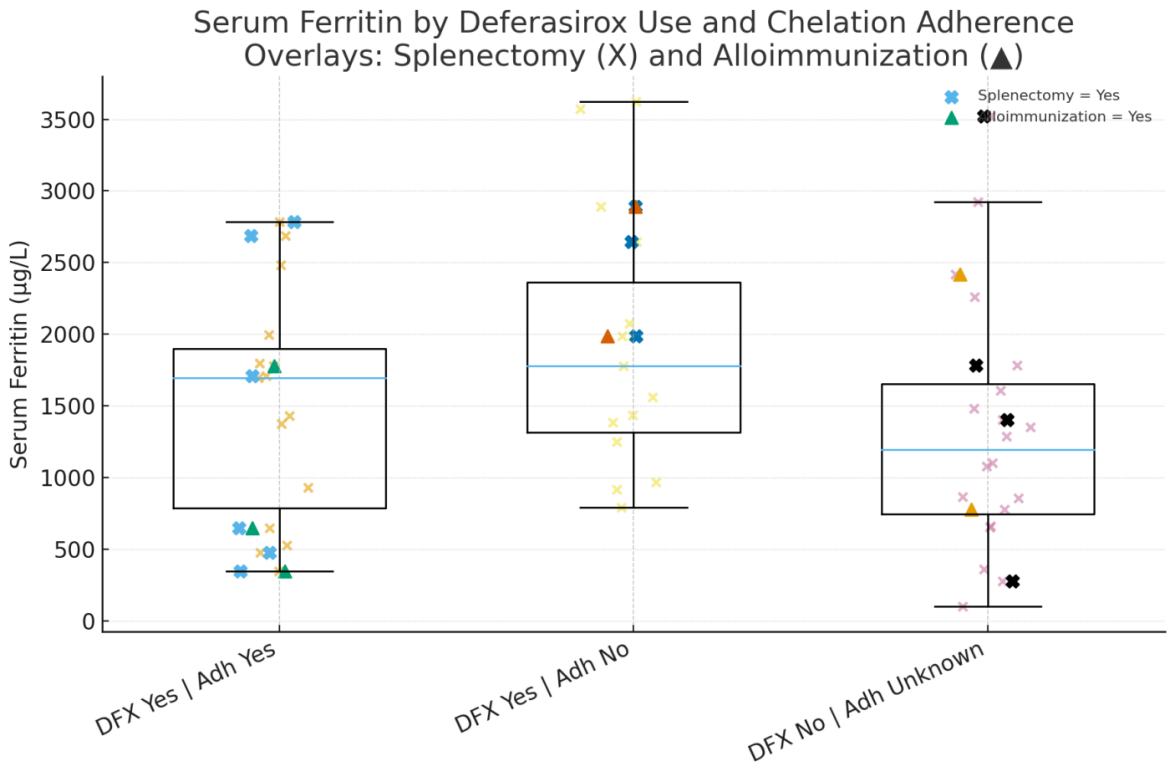

Fig 2: Overload Control in Practice: Serum Ferritin (µg/L) Distributions by Deferasirox Use and Chelation Adherence, with Splenectomy and Alloimmunization Overlays

This figure summarizes real-world ferritin control across chelation patterns. Boxplots show serum ferritin distributions for patients on deferasirox (DFX) with good adherence, DFX with poor adherence, and those not on DFX/unknown adherence. Jittered points depict individual patients; "X" marks splenectomized cases and "▲" marks alloimmunized cases. Median ferritin is lower in the DFX-treated groups, and the most favorable dispersion occurs with good adherence, indicating tighter iron control. Poor adherence shifts ferritin higher and broadens variability. Non-DFX/unknown patients display intermediate medians with wide spread. Overlays suggest splenectomy and alloimmunization cluster among higher ferritin values, highlighting clinically relevant risk concentrations.

Discussion

The present study demonstrated a substantial difference in transfusion needs and baseline hemoglobin levels between patients with severe versus mild β-thalassemia phenotypes. Patients with severe disease exhibited significantly lower pre-transfusion hemoglobin levels (6.9 ± 0.6 g/dL) and a markedly higher annual transfusion volume (188.5 ± 37.0 mL/kg/year), compared to their mild counterparts (9.9 ± 0.5 g/dL and 38.8 ± 20.5 mL/kg/year, respectively), with both comparisons reaching high statistical significance ($p < 0.001$). On a clinical level, these findings demonstrate the differences in transfusion burden in patients with more deleterious genotypes and decreased endogenous hemoglobin biogenesis, with support for stratified management according to genetic and hematologic severity.

These results are similar to those reported in the interim of the ULYSSES study in Greece in which the transfusion-dependent beta-thalassemia patients had a median pre-transfusion hemoglobin of 9.9 g/dL, which can be considered equivalent to the mild group in the current study. Moreover, according to the Greek cohort, the annulary transfusion level expressed as a mean number of red blood cell units was 34.9 units per year, corresponding to an estimated transfusion level in medium-to-severe disease, when converted to the mL/kg equivalent, according to body weight (Kattamis et al., 2020).

In addition, a 2023 study conducted in the US by Udeze et al reported that patients who were transfusion-dependent had an average single transfusion rate of 14.2 and pre-transfusion hemoglobin targets were around 9.5-10 g/dl. This reflects the trend towards mild phenotype seen in the present study, and indicates a worldwide trend of sustaining hemoglobin levels in the region of 10 g/dL for minimized disease burden (Udeze et al., 2023).

On the contrary, another view can be seen in data presented in a splenectomy based study in Bangladesh, where pre-splenectomy hemoglobin levels were considerably low, with a mean of 4.19 +- 0.92 g/dL. After splenectomy levels increased to 8.66 +- 0.73 g/dl and the need for transfusions decreased significantly. These results indicate that in certain clinical environments, the baseline hemoglobin values of the critically ill patients might be even lower than what has been seen in the present study, probably as a result of resource constraints or a different threshold of transfusion (Shahjahan & Elahi, 2025).

Consistent with the current observations, in a population-based study with 770,000 adult individuals in France, transfusion-dependent patients were averaged 13.5 transfusions per year and had higher mortality and complications, highlighting the clinical burden for high-volume transfusion. These data are consistent with the pattern of increased exposure to transfusion in the severe cohort of the current study (Baldwin et al., 2024).

A global review however by Forni et al (2023) considered a more nuanced view in which there is significant variation in transfusion practice depending on geographical and economic regions. Some regions have poor transfusion support, resulting in undertransfusion and falsely elevated haemoglobin levels in so-called mild cases. This finding adds an important caveat to the comparison of hemoglobin and transfusion data among populations and could partly account for any differences between studies (Forni et al., 2023).

In reconciling these findings, the consistency between the current study and several international datasets further supports the validity of the use of pre-transfusion Hb and annual transfusion volume as objective indicators of disease severity. The gradient of deviation in some cohorts can be due to variations of clinical infrastructure, transfusion regimes or adjuvant treatment such as splenectomy. The clinical implication is clear - standardizing transfusion thresholds and that of genetic profiling can help harmonize the classification of the disease globally. In the present study, the less severe patients with milder forms of Beta Thalassemia had significantly higher amounts of fetal hemoglobin (HbF.) (25.2 plus 11.9%) than those with severe disease (13.7 plus 7.6%), with a highly significant p-value of < 0.001. Moreover, hydroxyurea exposure was found to be more prevalent in the mild group (60%) than in the severe group (32%) but that difference was not statistically significant ($p = 0.089$). The clinical implications of such results indicate a correlation between an increase in HbF levels possibly mediated by hydroxorurea therapy and a less severe disease expression. This is in keeping with the well-documented mechanism of hydroxyurea as an HbF inducer and presumed ability to limit transfusion needs and disease severity.

These findings are broadly supported by a 2022 randomized controlled trial by Yasara et al., which demonstrated that hydroxyurea significantly increased HbF levels and reduced erythropoietic stress in β-thalassemia patients. Importantly, a subgroup of responders required less transfusion, particularly those with the HbE genotype and Xmn1 polymorphism, further highlighting the genotype-therapy interaction (Yasara et al., 2022).

Similarly, a prospective study by Fatima et al. in 2022 reported that hydroxyurea improved HbF levels significantly and also led to a notable reduction in transfusion frequency and symptoms of anemia, thus reinforcing its therapeutic potential in β-thalassemia major (Fatima et al., 2022).

Adding further support, Ghosh et al. (2021) explored the efficacy of hydroxyurea in patients with HbE/β-thalassemia and showed that patients carrying the XmnI polymorphism had significantly longer transfusion-free intervals and higher hemoglobin levels under HU therapy (Ghosh et al.,

2021).

In contrast, a 2024 study by Nawaz et al. found that although HbF was significantly associated with disease severity, neither HbF nor HbA2 levels correlated with treatment response categories to HbF-inducing therapies. This contradicts the present study's implication of HbF elevation correlating with milder disease and suggests a more complex, perhaps multifactorial, response profile in transfusion-dependent thalassemia patients (Nawaz et al., 2024).

Furthermore, Hatamleh et al. (2023) in a meta-analysis concluded that hydroxyurea use significantly prolonged transfusion intervals and increased HbF and hemoglobin levels across multiple studies, consolidating the drug's efficacy in improving clinical outcomes in transfusion-dependent β-thalassemia. This aligns well with the current study's observation of higher HbF and HU use in milder phenotypes (Hatamleh et al., 2023).

On the other hand, a recent study by Roy et al. (2025) focusing on NTDT (non-transfusion-dependent thalassemia) children reported that while the XmnI polymorphism predicted a better hydroxyurea response, the overall effect of HU was genotype-dependent, and its benefits were not universal. This variability suggests that hydroxyurea's utility may be restricted to certain genetic backgrounds, challenging the blanket generalizability of findings such as those in the present study (Roy et al., 2025).

These mixed findings indicate the importance of individualized genetic architecture on the determination of treatment response. The trend in the present study, although not statistically significant, for increased use of hydroxyurea in the mild group may reflect the increase in occurrence of favorable genotypes or better drug tolerance/adherence in the latter patients. On the other hand, patients with more severe disease may have genetic backgrounds that are less responsive to hydroxyurea and therefore contributed to lower HbF levels despite treatment.

In conclusion, the results present in the present study are highly concordant with recent literature displaying the role of hydroxyurea in increasing HbF and reducing severity of thalassemia. However, biological differences in genetic backgrounds, sample sizes and study designs may account for discrepancies among some studies. Future studies should focus on genotype-specific analysis and longer-term, multi-center studies that will help tailor the precision use of hydroxyurea for the treatment of beta thalassemia.

In the current study, serum ferritin concentrations in the patients with severe (2052.8 +-860.9 ug/L) and mild beta-thalassemia (1079.3 +-603.4 ug/L) were significantly higher ($p = <0.001$). The use of deferasirox was significantly higher in the severe group (80% vs. 40% $p = 0.009$), reflecting the higher iron burden requiring chelation. However, adherence to the chelation therapy was not different in the two groups, with only 50% of the groups considered to have had good adherence. These results highlight an important clinical observation, namely that even though chelation is prescribed more aggressively for patients with higher levels of iron, adherence is a persistent challenge, and may constrain the ability of chelation to be effective in both more severe and less severe cases.

This pattern of increased serum ferritin in more severely affected patients and an accompanying increase in chelation therapy are consistent with the results of several more recent studies. For instance, Shaker et al. reported results indicating that low adherence to deferasirox was significantly linked with high ferritin levels in a group of thalassemia patients and emphasized that even knowledge about the illness was significantly associated with levels of adherence (Shaker et al., 2024). Similarly, Mobinikhaledi et al. (2024) showed that better formulation of deferasirox (e.g., changing from Exjade(R) to Jadenu(R)) significantly improved patient's satisfaction and convenience which may lead to improved adherence and fewer complications concerning the iron overload (Mobinikhaledi et al., 2024).

Batool et al. in 2021 further supported the efficacy of deferasirox with significant decrease in serum ferritin over the course of 12 months which went from 4137 ng/ml to 2188 ng/ml and

supported the findings of the current study for efficacy of deferasirox in controlling iron overload when induce appropriately (Batool et al., 2021).

In contrast, Vagh et al. (2020) reported higher treatment reductions in serum ferritin (43.2%) using deferasirox/deferoxamine combination compared to monotherapy treatment (25.8%), and thus, monotherapy may not be optimal for some high-risk patients. This finding contradicts the implication in the current study for the use of deferasirox monotherapy for the management of severe iron overload, particularly in those with persistently high ferritin levels despite reported treatment.

Moreover, a study by Iram et al. (2024) suggested that there is no significant difference in ferritin reduction between deferasirox and deferoxamine in a large sample, which suggests that the choice of the chelator seems less impactful than adherence and dosing strategy (Iram et al., 2024). This is consistent with the focus in the present study on adherence versus chelator type as a limiting factor to reach target levels of ferritin.

Interestingly, Ricchi et al. (2010) reported a paradoxical case with significant rise in ferritin levels following the initiation of deferasirox therapy despite evidence of stability in the organ iron load using MRI (Ricchi et. al., 2010). This outlier indicates the complexity of relying on serum ferritin alone as an indicator of iron overload, and suggests issues in the current study's use of serum ferritin alone when adjunctive imaging studies (such as T2* MRI) should have been employed.

In synthesis, the present study's results on the relationship between the severity of the disease and serum ferritin, chelation usage and adherence have been supported in the recent literature. However, nuances arise in that the comparative efficacy of various chelation strategies, and the variable reliability of serum ferritin as a surrogate for total body iron. These findings collectively suggest that although deferasirox should continue to play a key role in iron chelation therapy, its optimal contribution to patient care will depend on formulation, adherence, and perhaps combination therapy in high burden cases.

The current study observed the high prevalence of splenectomy and alloimmunization in patients with b-thalassemia, with higher rates found in patients with a severe disease phenotype.

Splenectomy was more frequent among the severe group (40%) compared to the mild group (12%), with a p-value of 0.053, suggesting a borderline statistically significant difference. Alloimmunization was reported in 20% of the severe group and 8% of the mild group, though this difference did not reach statistical significance ($p = 0.415$). These trends align with clinical expectations, as patients with more severe disease often require higher transfusion volumes, predisposing them to alloimmunization and the consideration for splenectomy to manage transfusion burden and splenomegaly.

These findings are broadly supported by recent literature. For example, a study by Pazgal et al. (2020) in Israel reported that splenectomy was strongly associated with increased risk of alloimmunization in transfusion-dependent β-thalassemia patients, with a statistically significant correlation ($p = 0.016$) and a higher probability of alloantibody development in splenectomized individuals (Pazgal et al., 2020). Similarly, Minhas et al. (2022) found a significant association between splenectomy and RBC alloimmunization in a pediatric cohort with β-thalassemia, reinforcing the view that splenectomy contributes to heightened immune responses against transfused blood (Minhas et al., 2022).

Conversely, some studies have reported lower or non-significant associations. Arianezhad Eslamizadeh et al. (2025) in Iran found no significant difference in alloimmunization rates between splenectomized and non-splenectomized patients, suggesting that other factors—such as antigen matching or leukoreduction—may mitigate risks in well-managed settings (Arianezhad Eslamizadeh et al., 2025). Likewise, Almorish et al. (2024) in Yemen also did not find a statistically significant relationship between splenectomy and alloimmunization, despite

observing that most alloantibodies were against the Kell and Rh systems (Almorish et al., 2024).

Support for the association between splenectomy and increased alloimmunization was again reported in a large Egyptian cohort, where El-Beshlawy et al. (2020) demonstrated that both the presence of autoantibodies and alloantibodies were significantly associated with splenectomy status, duration of treatment, and transfusion frequency (El-Beshlawy et al., 2020). A more recent study by Aishwarya et al. (2024) in India found a statistically significant correlation ($p < 0.0001$) between alloimmunization and splenectomy, especially in patients with high transfusion loads, aligning well with the patterns noted in the present study (Aishwarya et al., 2024).

Taken together, the current study's observation that splenectomy was more frequent in patients with severe β-thalassemia is well-aligned with the prevailing literature, as these patients typically experience more aggressive disease requiring intensified transfusion support, which may lead to hypersplenism and necessitate splenectomy. The borderline significance ($p = 0.053$) observed may reflect sample size limitations rather than a lack of true effect. The trend toward increased alloimmunization in the severe group, although not statistically significant here, is consistent with the immunologic burden of frequent transfusions, further intensified in splenectomized individuals who lack the splenic filtering and immunomodulatory functions. Variation across studies could be attributed to differences in transfusion protocols, antigen-matching strategies, leukoreduction policies, and patient demographics, all of which modulate alloimmunization risk.

The present study identified a statistically significant difference in the distribution of β-globin genotypes between patients with severe and mild β-thalassemia phenotypes, with β^0/β^0 being far more prevalent in the severe group (68%) and β^+/β^+ being dominant in the mild group (60%) ($p = 0.000$). This confirms the well-established role of HBB mutation class in modulating disease severity, as β^0 alleles result in no β-globin production while β^+ alleles retain partial function. Though the differences in frequencies of fetal hemoglobin (HbF)-modifying alleles—namely XmnI (rs7482144), BCL11A (rs1427407), and HBS1L-MYB (rs9399137)—were not statistically significant, they trended toward enrichment in the mild group, consistent with their protective role.

These results align with several recent studies. For example, a 2023 study by Wong et al. showed that the presence of β^+ alleles and the rs9399137 C allele were significantly associated with milder hemoglobin E/β-thalassemia phenotypes in a Thai population. Their multivariate analysis found that rs9399137 alone reduced disease severity scores by more than three points, and the rs7482144 minor allele also showed significant impact (Wong et al., 2023).

Similarly, Shabaan et al. (2023) found that Egyptian children carrying protective variants at HBG2 (rs7482144), BCL11A (rs1427407), and HBS1L-MYB (rs9399137) exhibited delayed transfusion initiation and lower overall transfusion burden. The homozygous and heterozygous states of these polymorphisms were associated with higher fetal hemoglobin levels and lower Thalassemia Severity Scores (TSS), reinforcing the present study's suggestion that these variants, although not statistically significant in a small sample, may carry biological relevance in larger populations (Shabaan et al., 2023).

On the other hand, not all recent findings support the significant role of these modifiers. Walters et al. (2020), analyzing patients undergoing gene therapy, found no statistically significant association between any of the major HbF-modifying SNPs and transfusion independence outcomes. Although most patients carried these modifiers, their presence did not strongly predict treatment success, suggesting context-dependent modifier effects that may be overshadowed by more potent therapeutic interventions or gene therapy vectors (Walters et al., 2020).

Further contrasting findings come from a 2021 study by Bashir et al., which found that the HBS1L-MYB rs9399137 SNP had no significant influence on HbF levels in their β-thalassemia cohort, while XmnI (rs7482144) and BCL11A (rs766432) did. This partially supports the present study's findings in which rs9399137 did not reach significance, potentially due to sample size or

regional genetic variation (Bashir et al., 2021).

In contrast, a 2024 study by Al-Allawi et al. in Iraqi patients provided strong evidence for the predictive power of a genotype-based scoring system including β^+ alleles, rs7482144, and BCL11A rs1427407. Their logistic regression model achieved an AUC of 0.917, supporting the utility of combining these genetic factors for phenotype prediction—an approach closely mirrored in the composite genotype score used in the current study (Al-Allawi et al., 2024).

A particularly notable comparison arises from El-Ghamrawy et al. (2020), who observed that co-inheritance of multiple favorable HbF-raising SNPs had a stronger association with increased HbF and milder disease phenotype in Egyptian sickle cell disease patients, whereas individual SNPs did not have a consistent impact. This finding is mirrored in the present study, where no single modifier allele reached statistical significance, yet the overall trend favored cumulative protective effects in the mild group (El-Ghamrawy et al., 2020).

Taken together, these findings suggest that while the current study correctly identifies HBB genotype as a major determinant of thalassemia severity, the additive contribution of minor HbF modifier alleles—particularly when analyzed in aggregate—may require larger cohorts or population-specific models to reveal statistically robust associations. The observed non-significant tendencies towards enrichment of protective alleles in milder patients are in line with mechanistic expectations and should therefore not be dismissed, particularly since they are supported by robust findings in multiethnic studies as well as more population-specific prediction tools.

Conclusion

This study highlights the usefulness of combining genotypic and clinical data in a combined prediction model for estimation of the severity of v-thalassemia. A traumatic CGS, which combined both v-globin mutation class and fetal hemoglobin-modifying loci, demonstrated a good degree of power to distinguish between mild and severe phenotypes. Moreover, the Quantitative Severity Index (QSI), based on routine hematological parameters, provided an independent confirmation of disease burden, strongly discriminating the two groups in a clinically relevant way.

Interestingly, individual polymorphisms located in XmnI (HBG2), BCL11A, and HBS1L-MYB were not in themselves statistically significant given the sample size; however, their combined effect in the CGS was strongly predictive. This upholds the idea of genetic synergy, and facilitates acceptance of the clinical significance of these modifiers when evaluated together. Furthermore, the trends of hydroxyurea use and higher HbF levels in milder cases are biologically plausible correlations with treatment responsiveness and support the phenotype-genotype correlations.

The study's results serve to demonstrate the feasibility of a method of genotype-informed severity prediction that can be successfully applied in the resource-limited environment without costly imaging and biomarker panels. By turning genetic and hematologic data into operationalizations in the form of nomograms or severity indices, these will help clinicians to better stratify patients for intervention at an earlier stage, evaluate therapeutic effectiveness, and coincide respective transfusion regimens where necessary.

In conclusion, the CGS and QSI are useful and reproducible tools that meet the goals of precision medicine for Hbopathies internationally. Future studies in larger multi-ethnic cohorts are needed for further fine-tuning of these models and understanding genotype-therapy interactions, in which case, ultimately, optimizing of the individual care can be achieved in v-thalassemia.

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