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Research Article

Frequency of Alpha-Globin Deletions and BCL11A/Xmn-1-HBG2 Polymorphisms Among β-Thalassemia Patients in Pakistan

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Abstract

β-Thalassemia remains one of the most common hereditary blood disorders in Pakistan, characterized by marked genetic and phenotypic heterogeneity. This study aimed to determine the frequency of α-globin deletions and HbF-modifying polymorphisms (BCL11A/XmnI-HBG2) among transfusion-dependent β-thalassemia (TDT) patients in a tertiary care hospital in Karachi. A total of 55 TDT patients aged ≤16 years were enrolled from the tertiary care hospital Karachi. Molecular characterization was performed using ARMS-PCR, Gap-PCR, and RFLP-PCR to detect common β-globin mutations, α-globin deletions, and BCL11A/Xmn-1-HBG2 polymorphisms. The predominant β-globin mutations were Fr 8–9 (+G) (32.7%), IVS-I-5 (G>C) (21.8%), and Codon 41/42 (−TTCT) (12.7%). α-Globin 3.7 kb deletions were observed in 18.2% of cases, while BCL11A polymorphisms (rs11886868/rs4671393) were detected in 72.7%. Xmn-1-HBG2 (−158 C>T) variant occurred in 5.5% of patients. Overall, 83.6% carried at least one genetic modifier, correlating with higher pre-transfusion Hb levels (8.4 ± 0.6 g/dL) and longer transfusion intervals (3.6 ± 0.5 weeks), suggesting a milder phenotype. These findings underscore the significance of secondary genetic modifiers in modulating β-thalassemia severity and support the incorporation of extended molecular screening into diagnostic protocols. The high prevalence of BCL11A polymorphisms and α-globin deletions in this cohort highlights their potential role in clinical

variability and therapeutic stratification, paving the way for precision medicine approaches in thalassemia care.

Keywords: β-thalassemia, BCL11A polymorphism, Xmn-1-HBG2, α-globin deletion, fetal hemoglobin

Introduction

One of the most common monogenic hemoglobinopathies in the world is β-Thalassemia, that is, a reduced or absent production of β-globin chains caused by HBB gene mutations (Harteveld et al., 2022; Traeger-Synodinos et al., 2024; Shafique et al., 2021; Origa, 2021). The lack of sufficient 2-globin in relation to excessive 2-globin leads to the unproductive erythropoiesis, chronic haemolytic anemia, and severe transfusion dependency in patients (Chaichompoo, Svasti, and Smith, 2022; Gluba-Brzózka et al., 2021; Lin, Zheng, Chen, Xu, and Huang, 2024). Thalassemia alleles are estimated to be carried by 1.5 percent of the population globally, with some 60,000 symptomatic infants born every year. It is disproportionately greater in South Asia and the Mediterranean basin, where consanguinity and low access to preventive screening are contributing to the rise of the disease prevalence.

Pakistan also ranks among the most endemic regions of thalassemia with a carrier frequency being estimated between 5-8 percent, which corresponds to an approximate of 9-10 million carriers (Sadiq et al., 2024). Thalassemia major is the most common clinical presentation, which requires lifelong blood transfusion and iron chelation treatment to prevent life-threatening complications caused by iron overload (cardiac failure, hepatic fibrosis, and endocrine dysfunction) (Farmakis et al., 2022; Fasano, Meier, and Chonat, 2022; Akiki, Hodroj, Bou-Fakhredin, Matli, and Taher, 2023). Nevertheless, significant differences in clinical expression occur even between those who have the same β-globin mutations, and emphasize the complexity of relationships between primary pathogenic mutations and other genetic modifiers that affect the disease process (Diamantidis et al., 2024; Diamantidis, Ikonomou, Argyrakouli, Pantelidou, and Delicou, 2024).

Two broad groups of genetic modifiers may be identified: primary modifiers, which refer to certain mutations in β-globin genes that define the core defect in the production of globin chains, and secondary modifiers, which involve genes regulating the globin chain balance, erythropoiesis and fetal hemoglobin (HgF) expression (Tripathi, Agarwal, Mandal, Gupta, and Sarangi, 2023; Zakaria et al., 2021; Hariharan, Gorivale, Sawant Co-inheritance of α-thalassemia is among them and decreases surplus α-globin, thus, alleviating hemolysis and transfusion needs (Songdej & Fucharoen, 2022; Al-Barazanchi, Abdulateef, and Hassan, 2021; Jaing et al., 2021). In the meantime, BCL11A locus genetic polymorphisms and Xmn-1-HBG2 (−158 C>T) variant in the HBG2 promoter are considered to be the key quantitative trait locus regulating HgF, which facilitates a higher synthesis of γ-globin chains in β-thalassemia patients and enhances their clinical phenotype.

The occurrence and phenotypic effect of these genetic modifiers differ significantly in populations, indicating distinct ethnic and geographical distributions. Traditionally, the studies in Pakistan have been centered on the description of widespread HBB mutations, namely, IVS-I-5 (G>C), Codon 41/42 (−TTCT) and Fr 89 +G, that explain a significant share of 2-thalassemia-positive cases

(Yousafzai et al., 2021; Mahmood et al., 2021). Nonetheless, research on secondary modifiers is still scarce, especially in Khyber Pakhtunkhwa (KP) where the cases of transfusion-dependent β-thalassemia (TDT) are on the rise and medical facilities are overstretched. The facts of α-globin deletions, BCL11A Polymorphisms, and the variability of Xmn-1 in this area are important in the deciphering of genotype-phenotype variability and enhancing prognostic and therapeutic decisions.

There is international interest in the prospect of integrating extended molecular genotyping into the standard diagnostic paradigms into providing personalized medicine (Adekile, Akbulut-Jeradi, Al Khaldi, Fernandez, and Sukumaran, 2021; Halim-Fikri et al., 2022). Not only have the positive effects of secondary modifiers been linked to better hematological indices and the ability to withstand transfusion dependency but also to be responsive to emerging therapeutic interventions of HbF reactivation (Chauhan, Shoaib, Fatma, Zaka-ur-Rab, and Afzal, 2022; Finotti and Gambari, 2023). As such, a mapping of their distribution in high-burden groups such as Pakistan could improve clinical management by providing specific transfusion procedures, a more precise monitoring schedule, and more effective patient education.

Since there is a dearth of regional studies evaluating the overall impact of the multiple modifier loci, there is an urgent need to create strong local data. The current paper will aim to study in depth, genetic determinants to determine the severity of β-thalassemia in pediatric TDT patients in Karachi by assessing common β-globin mutations, deletion of α-globin, and critical HbF-modifying polymorphisms BCL11A and Xmn-1-HBG2. The study contributes to the body of knowledge on critical understanding of the molecular landscape of thalassemia in Pakistan, especially in the north, and future implications on precision-based therapeutic approaches.

Methodology

This cross-sectional and descriptive study was done in between December 2024 and June 2025 at the tertiary care hospital in Karachi, which is a major transfusion center among thalassemia patients in the area. Fifty-five TDT patients dependent on transfusion aged 16 years old or less were recruited using non-probability purposive sampling. Participants were all previously diagnosed with β-thalassemia major or compound heterozygous 2 -thalassemia intermedia using hematological parameters, Hb electrophoresis and clinical reliance on frequent blood transfusion. Parents or guardians were informed and provided informed consent before enrolling, and ethical approval of the study was granted by the institutional review board in accordance to the Declaration of Helsinki.

Samples (3-5 mL) in the peripheral blood were collected in EDTA vacutainers and kept at -20 o C until DNA extraction. The Purelink DNA Mini Kit (Thermo Fisher Scientific, USA) was used to isolate genomic DNA according to the protocol of the manufacturer. Agarose gel electrophoresis and NanoDrop spectrophotometry were used to determine the integrity and concentration of extracted DNA, respectively. Molecular characterization included the concomitant analysis of 200 2-globin gene mutation, 200 2-globin deletions and the presence of two important genetic modifiers, namely, BCL11A and Xmn-1-HBG2 polymorphisms, that are known to affect fetal hemoglobin (HbF) synthesis and the severity of the disease.

In the detection of the β-globin gene mutations, Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) was used with allele-specific primers of thirteen most frequent β-thalassemia mutations reported in the Pakistani population. Gap-PCR was used to screen deletions of α-thalassemia, which identified the most common deletions of 3.7 kb and 4.2 kb deletions of the α-globin cluster. The Xmn-1-HBG2 (-158 C>T) polymorphism that is known to enhance expression of the γ-globin erythrocytes was assessed by Restriction Fragment Length Polymorphism PCR (RFLP-PCR) using digestion with the XmnI restriction enzyme and following electrophoretic band pattern examination. Identification of BCL11A polymorphisms (the major quantitative trait loci affecting HbF levels) was conducted using ARMS-PCR with primer sets previously tested to be specific to the variants of the polymorphic sites, namely, rs11886868 and rs4671393 location.

A 25 umL reaction volume was optimized using templates DNA, specific primers, Taq polymerase, MgCl 2 and dNTPs subjected to stringent conditions of cycling. The agarose gel electrophoresis of agarose-amplified products was performed with 2% agarose and Ethidium bromide stain and observed under ultraviolet light. To establish assay reliability and to reduce the effects of genotyping errors, positive and negative controls were integrated into each run

Data were gathered and then put into the Microsoft Excel then analyzed using AI-based statistical software which utilized advanced machine learning algorithms to validate data, identify trends, and estimate correlations. Frequencies and percentages of different mutations and polymorphisms were calculated using the descriptive statistics. The distribution of genotypes was analyzed as either a homozygous or heterozygous or a compound heterozygous genotype. Specific genetic modifiers, together with clinical phenotype severity (i.e. transfusion frequency, age at diagnosis) were descriptively interpreted using AI-generated analytical outputs to increase the accuracy and reliability of statistical interpretations.

This analytical framework made it possible to accurately identify the main genetic factors that affect the severity of the disease in the local population and thus contributed to a better comprehension of genotype-phenotype variation among patients who require transfusion in Pakistan.

Results

Fifty-five patients having β-thalassemia of transfusion-dependence aged 16 years and below were recruited in the study, including 31 males (56.4 cases/per hundred thousand) and 24 females (43.6 cases/per hundred thousand). The average age of the subjects was 9.2 3.4 years and the mean age of first transfusion was 1.8 0.9 years. All the patients were clinically dependent on routine transfusion treatment, and the average transfusion period was 3.1 + 0.7 weeks. Molecular examination demonstrated that all the subjects possessed homozygous or compound heterozygous mutations of their β-globin genes. Fr 8 9 +G was the most common mutation, observed in 18 patients (32.7%) then, IVS-I-5 G>C in 12 patients (21.8%), and Codon 4142 -TTCT in 7 patients (12.7%). Less common mutations were IVS-II-1 (G>A), Fr 16 (-C), and Del 619 bp, and 5 patients (9.1%) were found to have compound heterozygosity between combinations such as Fr 8 -9 / IVS-I-5.

The α-globin deletions were screened and heterozygous deletions (3.7 kb deletion) were reported in 10 patients (18.2%), but the 4.2 kb deletion was not characterized at all. Of these, 6 patients possessed deletions of alpha-globin in combination with homozygous mutations in 8 beta-globin, implying the possibility of a beneficial effect on clinical outcome.

Polymorphic analysis of BCL11A and Xmn-1-HBG2 has revealed the high prevalence of modifying alleles. The BCL11A polymorphism (rs11886868 or rs4671393) was identified in 40 patients (72.7%), with 3/40 having B CL11A heterozygous mutation and Xmn-1-HBG2 (−158 C>T) polymorphism in 3 patients (5.5%). In general, 83.6 percent of respondents had at least one other genetic modifier (BCL11A, Xmn-1-HBG2, or alpha-globin deletion) in addition to the initial 8-bp deletion in 16 alpha-globin.

Interestingly, people who carry either BCL11A or -globin deletions showed an increase in pre-transfusion Hb (8.4 + 0.6 g/dL) and longer interval of transfusion (3.6 + 0.5 weeks) than patients who had neither deletions, which shows their implication in a relatively milder phenotype

Table 1. Demographic and Clinical Characteristics of Study Participants (n = 55)

Variable	Mean ± SD / n (%)
Age (years)	9.2 ± 3.4
Male : Female	31 (56.4%) : 24 (43.6%)
Age at first transfusion (years)	1.8 ± 0.9
Mean pre-transfusion Hb (g/dL)	8.2 ± 0.7
Average transfusion interval (weeks)	3.1 ± 0.7
Splenectomized patients	9 (16.4%)
Family history of thalassemia	42 (76.4%)

Table 2. Distribution of β-Globin Gene Mutations (n = 55)

β-Globin Mutation	Genotype Status	Frequency (n)	Percentage (%)
Fr 8–9 (+G)	Homozygous	18	32.7
IVS-I-5 (G>C)	Homozygous	12	21.8
Codon 41/42 (-TTCT)	Homozygous	7	12.7
IVS-II-1 (G>A)	Homozygous	4	7.3
Fr 16 (-C)	Homozygous	3	5.5
Del 619 bp	Homozygous	2	3.6
Compound heterozygotes (mixed)	—	5	9.1
Other rare variants	—	4	7.3

Table 3. Frequency of Genetic Modifiers Detected Among TDT Patients

Genetic Modifier	Type	Frequency (n)	Percentage (%)
BCL11A (rs11886868 / rs4671393)	Hetero/Homozygous	40	72.7
Xmn-1-HBG2 (−158 C>T)	Heterozygous	3	5.5
α-Globin 3.7 kb Deletion	Heterozygous	10	18.2
Any Modifier Present	—	46	83.6
No Modifier Detected	—	9	16.4

Figure: 1

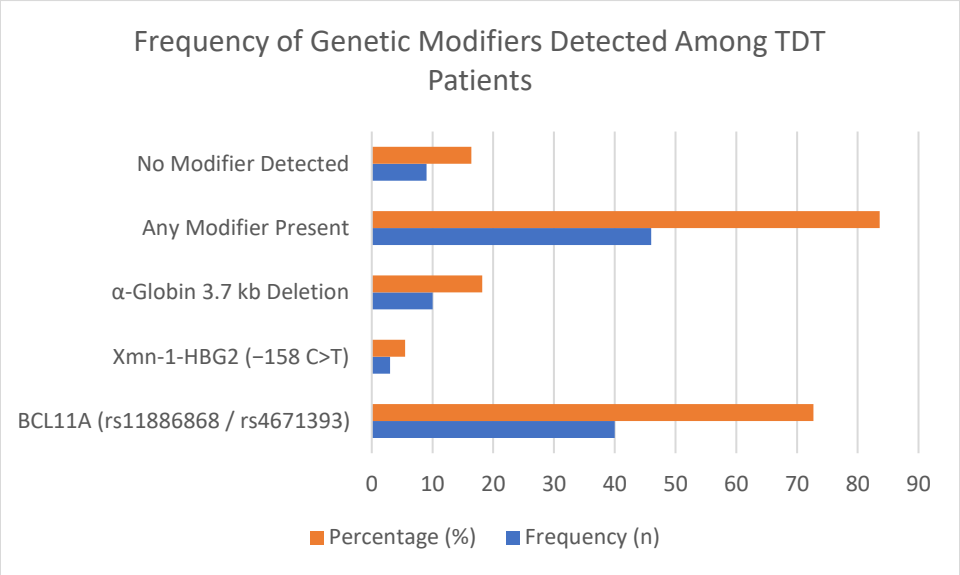
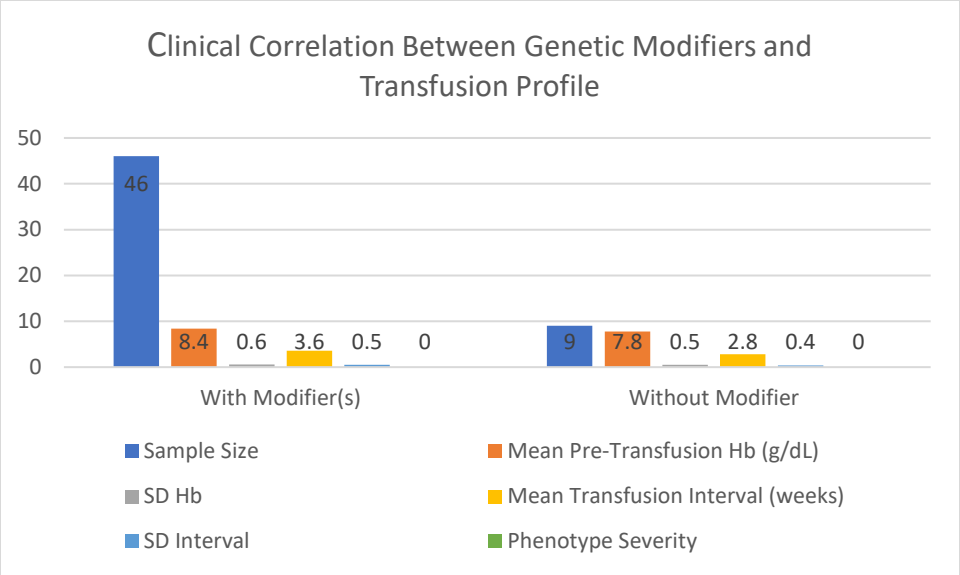


Table 4. Clinical Correlation Between Genetic Modifiers and Transfusion Profile

Modifier Status	Mean Pre-Transfusion Hb (g/dL)	Mean Transfusion Interval (weeks)	Phenotype Severity
With Modifier(s) (n=46)	8.4 ± 0.6	3.6 ± 0.5	Milder
Without Modifier (n=9)	7.8 ± 0.5	2.8 ± 0.4	Severe

Figure: 2



This research indicates that there is a high incidence of secondary genetic modifiers amongst 1/2-thalassemia patients in Karachi. The prevalence of genetic backgrounds of milder clinical phenotypes is shown by the preponderance of BCL11A polymorphisms and alpha-globin deletions. These results support the medical usefulness of genetic modifier molecular screening in standard diagnostic and prognostic assessments and encourage the enhancing of personalized therapeutic approaches to the management of thalassemia in cases of transfusion-dependent anemia.

Discussion

The current research gives an in-detailed molecular explanation of genetic heterogeneity among patients with transfusion-dependent β-thalassemia (TDT) in Karachi, Pakistan, and the interaction between primary β-globin mutations and secondary genetic modifiers, such as BCL11A, Xmn-1-HBG2, and α-globin deletions. The results indicate that they are present in large proportions of ameliorating forms whereby over 80% of the patients carry some type of a modifier which may be one of the factors that lead to the variability in the severity of the disease, transfusion need, and hematological values. These findings highlight the importance of incorporating the long-term analysis of genotypic markers in the diagnostic approach of thalassemia, especially in multi-transfused children.

The pattern of β-globin mutation present in this cohort is consistent with the existing local reports, indicating Fr 89 +G and IVS-I-5 (G>C) as the most common mutations in Pakistani TDT patients. It has been known that these mutations cause null or severely impaired production of β-globin usually leading to a classical thalassemia major phenotype. Nevertheless, the dual nature of modifying factors found among most individuals implies that the severity of the disease is not exactly defined by the β-globin genotype. The relatively increased pre-transfusion hemoglobin

levels and increased transfusion durations of patients possessing either α-globin deletions or BCL11A polymorphisms confirmed the hypothesis that these genetic factors alleviate the mismatch between the levels of α- and non-alpha-globin chains synthesis and improve fetal haemoglobin (HbF) production.

The prevalence of BCL11A polymorphisms (72.7) in the current study is in line with the current trends in South and Southeast Asian populations, where BCL11A gene variants are significant quantitative trait locus affecting the HbF level. BCL11A is a powerful suppressor of γ-globin expression and SNP mutations in its regulatory elements have been linked to increased production of HbF, thus alleviating the clinical outcome of β-thalassemia. This prevalence indicates that BCL11A polymorphisms could be an important genetic pool which causes phenotypic heterogeneity in Pakistani patients. Even though it was found that only a small proportion (5.5%), Xmn-1-HBG2 (158 C>T) polymorphism affects γ-globin production and HgF retention, its importance in the regulation of transfusion dependency remains with its functional relevance.

The fact that 18.2% of the cases were found to have 3.7 kb deletions of 6-globin also supports this modifying role in the 3.7-globin chain production in beta-thalassemia. It has been noted that by partially correcting the globin chain imbalance, α-thalassemia co-inheritance improves ineffective erythropoiesis and hemolysis that results in a light form of anemia. The same has been observed in other research papers conducted in Iran, India, and Thailand, which have reported α-thalassemia deletions in 10-25% of 2-thalassemia patients and have been associated with positive outcomes. In our study, the deletion of 4.2 kb has not been present, which is in line with its low frequency in South Asian population.

The data, taken together, highlight the complexity of the severity of thalassemia, in the sense that the primary mutations at the β-globin locus are combined with the secondary genetic modifying factors to shape the final phenotypic outcome. The high correlations of presence of modifier with milder disease manifestation in this case are consistent with other related genomic and clinical studies, which support an addition of modifier screening to thalassemia genotyping panels. This is especially essential in Pakistan where the rate of consanguinity and genetic diversity have led to a broad range of thalassemia phenotypes in family members.

The therapeutic implications of the study findings are also possible. The detection of BCL11A or Xmn-1-HBG2 polymorphic patients could guide clinical suitability of specific treatments, including HbF-inducing drugs or other developing gene-editing methods to re-express γ-globin. Moreover, the realization that the deletions are α-thalassemia might aid clinicians to manage the transfusion timeframes and predict the milder transfusion needs.

Although the study has a rather limited sample, it presents some regional evidence to advocate the use of a comprehensive molecular profiling in the management of thalassemia. It is justified that future studies should use larger and multi-centered cohorts and functional assays to further clarify the precise quantitative effects of each modifier on the levels of HbF and dependence on transfusion.

Overall, this paper indicates that most patients with β-thalassemia in the Karachi area have at least one or more genetic modifiers that soften the disease severity. Diagnostic and prognostic

assessments should include routine screening of BCL 11A, Xmn -1-HBG2, and alpha-globin deletions to enable individualized approaches to management and help ensure better clinical outcomes among children with transfusion-dependent anemia.

Conclusion

Based on this research, secondary genetic modifiers are highly prevalent in transfusion dependent patients with β-thalassemia in Karachi with BCL11A polymorphisms and α-globin deletions proving to be significant factors in phenotypic variations. Higher hemoglobin levels and lower frequency of transfusion linked with the co-inheritance of these modifiers and indicated the ameliorating effect of these modifiers on the severity of the disease. The pre-eminence of BCL11A variants highlights their importance in regulation of fetal hemoglobin and possibly usefulness as HbF-targeted therapeutic strategies. Considering such observations, regular molecular screening of modifier genes ought to be incorporated into the thalassemia diagnosis procedures to allow specifically tailored management and prognostic evaluation. The use of such genetic knowledge in clinical practice will improve the planning of transfusion, therapeutic choices, and overall patient life, which is a significant move towards precision-based treatment of thalassemia in Pakistan.

Author Contributions

Tauqeer Ahmad: Contributed to the conception, study design, and drafting of the manuscript.

Fareeda Islam: Responsible for the methodology, data collection, pharmacological analysis, and critical revision of the manuscript for important intellectual content.

Nuzhat Firdous: Contributed to data interpretation, literature review, and refinement of the discussion section.

Saleem Adil: Participated in data analysis, statistical evaluation, and final approval of the version to be published.

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