Case Report

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2024.60.009384

Genetic and Clinical Interplay in Hematologic Disorders: A Case Study on Overlapping Anemia Etiologies

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ARTICLE INFO

Received: December 11, 2024

Published: December 17, 2024

Citation: Ali Torabi, Zeynal Sütkurt, Feyza Mermer, Ebru Marzioğlu Özdemir and Özkan Bağcı. Genetic and Clinical Interplay in Hematologic Disorders: A Case Study on Overlapping Anemia Etiologies. Biomed J Sci & Tech Res 60(1)-2024. BJSTR. MS.ID.009384.

ABSTRACT

Diagnosing patients with overlapping cytopenias requires a nuanced approach, particularly when clinical and genetic findings intersect. This case study explores a 22-year-old female presenting with anemia, thrombocytopenia, and bleeding tendencies. Comprehensive genetic analysis revealed a heterozygous CD36 variant contributing to macrothrombocytopenia and a delta-beta thalassemia-associated HBB variant. While the HBB heterozygous variant influences anemia profiling, it does not fully explain the severity observed. Chronic blood loss due to persistent bleeding tendencies emerges as the primary etiology, supported by laboratory findings of low hemoglobin, elevated Total Iron-Binding Capacity (TIBC), and reduced free iron levels. These findings underline the diagnostic challenge posed by the interplay of inherited and acquired factors. Additionally, next-generation sequencing identified somatic mutations in DNMT3A and ASXL1 in bone marrow evaluation, hallmark features of clonal hematopoiesis, suggesting an early-stage Myelodysplastic Syndrome (MDS). A germline MUTYH mutation further increased susceptibility to hematologic malignancies, highlighting the complexity of this case. This report underscores the importance of integrating somatic and germline findings in hematologic disorders. By distinguishing between independent etiologies, we demonstrate the clinical value of genetic profiling in guiding personalized management strategies for complex cytopenia.

Keywords: Bicytopenia; Semi Dominant; CD36 Mutation; Chronic Blood Loss Anemia; Clonal Hematopoiesis; Macrothrombocytopenia

Abbreviations: TIBC: Total Iron-Binding Capacity; MDS: Myelodysplastic Syndrome; RDW: Red Cell Distribution Width; ARPTT: Activated Partial Thromboplastin Time; PT: Prothrombin Time; FISH: Fluorescence *In Situ* Hybridization; NDS: Next-Generation Sequencing; CHIP: Clonal Hematopoiesis of Indeterminate Potential; CCUS: Clonal Cytopenia of Undetermined Significance; VEP: Variant Effect Predictor; MCH: Mean Corpuscular Hemoglobin

Introduction

Evaluating patients with cytopenias often presents a diagnostic challenge, particularly when multiple findings overlap, creating a complex clinical picture. These cases demand careful integration of clinical judgment, laboratory evaluations, and advanced genetic analysis. Here, we present the case of a 22-year-old female with concurrent anemia, thrombocytopenia, and bleeding tendencies, including spontaneous hematomas, hematochezia, and gingival bleeding. These symptoms initially suggested a bleeding disorder, yet the co-occurrence of anemia and thrombocytopenia posed an additional diagnostic dilemma: Are these findings manifestations of a single underlying disorder or independent entities with distinct etiologies? Genetic testing revealed a heterozygous CD36 variant, a known cause of macrothrombocytopenia in recessive cases, and a delta-beta thalassemia-associated HBB heterozygous variant, contributing to the patient's complex hematologic profile. While the HBB variant influenced the anemia profile, it could not alone account for the severity observed, which was primarily driven by chronic blood loss.

 DOI: 10.26717/BJSTR.2024.60.009384

This dynamic underscores the diagnostic difficulty posed by inherited conditions that modulate laboratory findings without being primary etiologies. Laboratory results, including reduced hemoglobin, elevated Total Iron-Binding Capacity (TIBC), and low free iron levels, support bleeding-induced anemia compounded by the genetic profile (Figure 1). Furthermore, somatic mutations in DNMT3A and ASXL1 were detected, hallmark features of clonal hematopoiesis, indicating early-stage Myelodysplastic Syndrome (MDS). A germline MUTYH mutation, associated with increased susceptibility to hematologic malignancies, added another layer of complexity and risk to the patient's clinical course. This case highlights the interplay between genetic predisposition and acquired factors in hematologic disorders. By presenting this patient's clinical and molecular findings, we aim to [1] explore the challenges of distinguishing between single and dual etiologies in cytopenias, [2] examine the contributions of germline mutations to complex phenotypes, and [3] emphasize the role of advanced genetic profiling in guiding personalized management strategies.

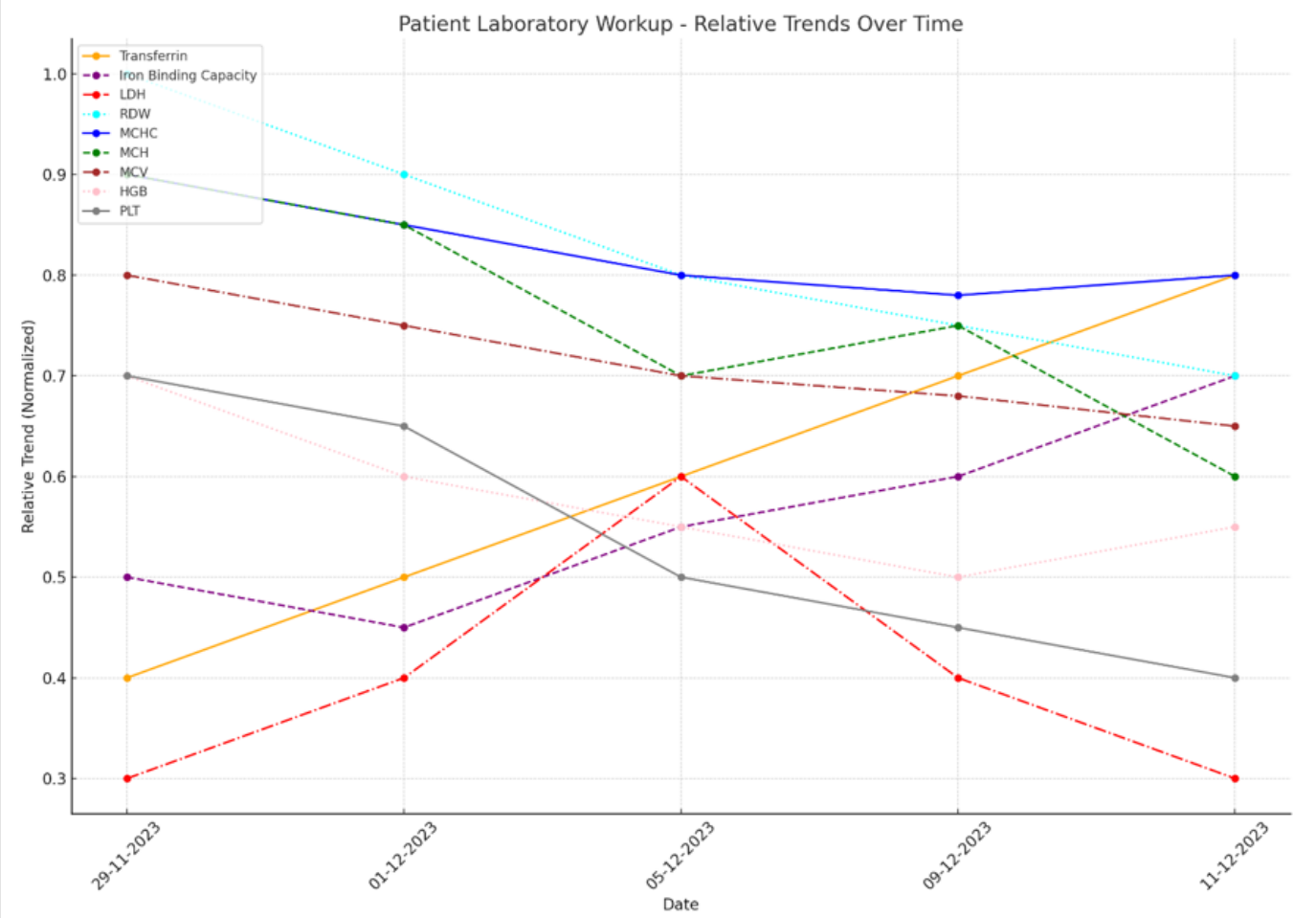

Figure 1: This figure illustrates the normalized trends of key laboratory parameters for the patient across five time points. Iron studies, including transferrin and Total Iron-Binding Capacity (TIBC), show a progressive rise, consistent with iron deficiency anemia secondary to chronic blood loss. Hemoglobin (HGB) and platelet count (PLT) display a declining trend, correlating with worsening anemia and thrombocytopenia. Red cell distribution width (RDW) remains elevated, reflecting anisocytosis, a hallmark of mixed anemia profiles. Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), and Mean Corpuscular Hemoglobin (MCH) display relatively stable yet slightly declining patterns, indicative of the combined effects of iron deficiency and delta-beta thalassemia. Lactate Dehydrogenase (LDH) trends upward, suggesting increased hemolysis or tissue turnover. These dynamic shifts underscore the interplay between chronic bleeding, genetic contributions, and evolving hematologic changes in the patient's clinical course.

Case Report

A 22-year-old female presented with recurrent spontaneous bleeding episodes, including hematomas, hematochezia, and gingival bleeding, first noted during childhood. Despite intermittent thrombocytopenia documented in her pediatric follow-ups, no definitive diagnosis was established. The patient reported no history of anticoagulant or antiplatelet use and denied any chronic medication use linked to bleeding disorders. The patient also reported a history of chronic diarrhea evaluated at 12 years old, with negative results for parasitic infections and celiac disease. At that time, mild anemia was noted, characterized by increased Red Cell Distribution Width (RDW), reduced hemoglobin (9 g/dL), and normal platelet counts. Despite her bleeding tendencies, no complications were reported during her pregnancy, which ended in a cesarean section due to dystocia.

On her recent presentation, physical examination was unremarkable except for hepatomegaly identified via abdominal ultrasound. Laboratory investigations revealed thrombocytopenia (platelet count: 146 × 10^9/L), anemia (hemoglobin: 9 g/dL), and elevated mean platelet volume (MPV: 13.6 fL) suggested platelet activation or turnover, likely secondary to macrothrombocytopenia. Iron studies demonstrated low free iron levels, elevated transferrin, and Total Iron-Binding Capacity (TIBC), consistent with iron deficiency (Figure 1). Vitamin B12 and folate levels were significantly reduced. Urinalysis revealed microalbuminuria and pyuria, suggesting a potential underlying renal issue. Coagulation studies, including Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT), von Willebrand factor levels, and activity, were within normal limits. Given the persistent cytopenias, a differential diagnosis of Myelodysplastic Syndrome (MDS) was considered. Bone marrow aspiration and conventional cytogenetic analysis revealed no dysplasia or chromosomal abnormalities. Flow cytometry findings (Table 1) supported the absence of overt hematologic malignancy. These inconclusive findings prompted referral to the medical genetics department for further evaluation, including Next-Generation Sequencing (NGS), germline testing, and Fluorescence in Situ Hybridization (FISH).

Table 1: Flow Cytometry analysis results.

Marker	Result	Description
Glycophorin A	Positive (50%)	Marker for erythroid cells, indicating the presence and maturation of red blood cell precursors.
MPO (Myeloperoxidase)	Positive (98.4%)	Enzyme marker for myeloid lineage cells, helps identify granulocytes and can indicate acute myeloid leukemia (AML) in high amounts.
CD61	Positive	Marker for megakaryocytes (platelet precursors), typically used in diagnosing myeloid disorders involving platelets.
CD34	Negative	Stem cell and progenitor cell marker, often used to identify blasts in leukemia or assess marrow cellularity.
bcl-2	Positive (10%)	Anti-apoptotic marker, commonly expressed in various cell types, indicating cell survival; overexpression seen in some malignancies.
CD3	Positive (15%)	T-cell marker, indicating the presence of T-lymphocytes in the sample.
CD5	Positive (15%)	T-cell marker, also present on some B-cells in disorders like chronic lymphocytic leukemia (CLL).
CD10	Positive (50%)	Marker for immature lymphoid cells and follicular center cells, sometimes associated with lymphoblastic leukemia/lymphoma.
CD20	Positive (1%)	B-cell marker, indicates presence of mature B-lymphocytes.
PAX-5	Positive (10%)	B-cell lineage marker, helps in identifying B-cell lymphocytes and certain lymphomas.
CD23	Negative	Marker often used to characterize B-cell chronic lymphocytic leukemia (CLL) and other B-cell lymphoproliferative disorders.
CD43	Positive	Marker found on T-cells, myeloid cells, and activated B-cells; used in lymphoid malignancy differentiation.
Cyclin D1	Negative	Cyclin D1 staining can be used in immunohistochemistry to help diagnose certain cancers, especially lymphomas, by confirming the presence of abnormal cell cycle activity.
CD33	Positive (34.6%)	Marker for myeloid cells, frequently expressed in acute myeloid leukemia (AML) and used to evaluate myeloid differentiation.
CD56	Negative	Neural cell adhesion molecule, seen in NK cells and also aberrantly expressed in some hematologic malignancies like AML and plasma cell myeloma.
CD117 (c-Kit)	negative	Stem cell factor receptor, often found on early hematopoietic progenitor cells and some myeloid leukemia cells.
CD79a	Positive (5%)	B-cell marker, used alongside CD20 to identify mature B-cells.
TRAP	Negative	Tartrate-resistant acid phosphatase, typically found in osteoclasts and hairy cell leukemia.
TdT	Positive (2%)	Terminal deoxynucleotidyl transferase, a marker for immature lymphoid cells, frequently used in diagnosing lymphoblastic leukemia/lymphoma.

Results

Advanced molecular and genetic testing was conducted to investigate the etiology of the patient's hematologic abnormalities. Next-Generation Sequencing (NGS) of bone marrow aspirates identified somatic mutations in DNMT3A and ASXL1, genes commonly associated with clonal hematopoiesis. These mutations are hallmark features of conditions such as Clonal Hematopoiesis of Indeterminate Potential (CHIP) and Clonal Cytopenia of Undetermined Significance (CCUS), and they suggest a potential for progression to Myelodysplastic Syndrome (MDS). Complementary Fluorescence in Situ Hybridization (FISH) analysis showed no chromosomal rearrangements or deletions, including abnormalities in 5q or 7q regions typically seen in MDS. Germline genetic analysis revealed three significant findings. A heterozygous CD36 mutation (ENST00000447544.7.1079T>G, p.Leu360*), traditionally linked to macrothrombocytopenia in recessive cases. The observed variant is hypothesized to exhibit a semi-dominant inheritance pattern, contributing to platelet dysfunction and mild thrombocytopenia in heterozygous individuals. A delta-beta thalassemia-associated HBB variant in its heterozygous form (ENST00000335295.4.208G>A) partially explained the anemia phenotype. This variant contributed to microcytosis and increased Red Cell Distribution Width (RDW) but did not account for the severity of anemia observed in this case.

A germline MUTYH mutation (ENST00000456914.7.1353_1355del), a gene critical for base excision repair. This mutation is associated with an increased predisposition to hematologic malignancies and likely contributed to the patient's susceptibility to clonal hematopoiesis. Complementary fluorescence in situ hybridization (FISH) analysis was performed to assess for chromosomal abnormalities commonly associated with hematologic malignancies. The results showed no evidence of rearrangements or deletions, including the 5q or 7q abnormalities often observed in MDS. Flow cytometry analysis (Table 1) highlighted several key findings, including markers indicative of erythroid and myeloid differentiation, without evidence of significant dysplasia or overt malignancy. Positive markers such as CD61 (megakaryocyte lineage) and MPO (myeloid lineage) supported the functional presence of precursor cells, while the absence of CD34 and CD117 expression indicated a lack of immature progenitor cell expansion. Collectively, these findings emphasized the complexity of the patient's hematologic abnormalities, integrating somatic and germline variants with clinical and laboratory data to delineate independent etiologies for thrombocytopenia and anemia.

Discussion

This case highlights the diagnostic complexity in patients presenting with overlapping hematologic abnormalities, particularly when multiple genetic findings interplay with clinical phenotypes. The concurrent anemia and thrombocytopenia initially suggested a unifying etiology, such as early-stage myelodysplastic syndrome (MDS). However, advanced investigations revealed distinct genetic contributions to each cytopenia, challenging the conventional diagnostic framework and underscoring the importance of precise molecular characterization. A significant finding, in this case, was the heterozygous CD36 mutation (ENST00000447544.7.1079T>G, p.Leu360*), a stop-gain variant known to result in a truncated CD36 protein. Traditionally associated with recessive inheritance and macrothrombocytopenia, this variant offers novel insights into phenotypic variability. Our observation supports the hypothesis that the mutation may exhibit a semi-dominant effect, contributing to platelet dysfunction even in heterozygous carriers. While CD36 deficiency has been linked to metabolic and cardiovascular susceptibilities such as coronary artery disease and insulin resistance [1-8], its role in platelet dysfunction as a trait-like feature remains underexplored [9-11]. This case is the first to propose a contribution of a heterozygous CD36 mutation to thrombocytopenia, expanding the phenotypic spectrum and inheritance model of this gene. The functional significance of this mutation is further supported by computational tools such as the Variant Effect Predictor (VEP) and Franklin, which classified the variant as pathogenic. Key ACMG/AMP criteria reinforce its pathogenicity, including PVS1 (Very Strong) for loss-of-function mutations and PM2 (Moderate) for rarity in population databases.

The genomic constraint analysis of the region utilizing the gnomAD browser surrounding the CD36 variant (chr7-80301310 T>G, p.Leu360*) revealed a negative Z score (-4.68), indicating a higher-than-expected number of observed variants. This suggests that the region is under relaxed evolutionary constraint, consistent with the presence of polymorphisms or trait-associated variants rather than exclusively severe pathogenic mutations. However, the specific stop-gain mutation identified in this case introduces a loss of function, as supported by the PVS1 criterion, overriding the general tolerance of variation in the region. This dual perspective raises the possibility that the CD36 variant may act as both a trait-contributing polymorphism and a pathogenic factor, depending on zygosity and genetic or environmental context. These findings further support the hypothesis that the CD36 mutation may contribute to thrombocytopenia in a semi-dominant manner, expanding the phenotypic spectrum and inheritance model of CD36-related disorders.

Another critical finding was the heterozygous delta-beta thalassemia-associated HBB variant (ENST00000335295.4.208G>A), which influenced the patient's anemia profile. In heterozygous states, this variant is typically associated with subtle changes in red cell indices, such as reduced Mean Corpuscular Volume (MCV), increased red cell distribution width (RDW), and slightly altered Mean Corpuscular Hemoglobin (MCH), rather than severe anemia. In this case, while the variant contributed to the hematologic presentation by modifying the red blood cell profile, it alone could not explain the patient's pronounced anemia. The primary driver of the patient's significant hemoglobin and iron declines appeared to be chronic blood loss due to persistent bleeding tendencies. This was supported by laboratory findings, including elevated Total Iron-Binding Capacity (TIBC),

depleted iron stores, and persistently low hemoglobin levels. The HBB variant complicated the anemia profile by overlapping with and masking features of iron deficiency anemia, which posed challenges in establishing a clear differential diagnosis.

The interplay between the genetic contribution of the HBB variant and the acquired iron deficiency underscores the complexity of this case. Furthermore, the mild increase in lactate dehydrogenase (LDH) likely reflected a secondary effect of chronic blood loss and hemolysis, driven by accelerated red blood cell turnover. This dynamic interplay highlights the necessity of considering both inherited factors and acquired conditions when evaluating patients with multifactorial hematologic abnormalities. This dual diagnostic approach avoids oversimplification under a single disease entity and enables targeted management strategies tailored to each condition. Notably, while the HBB variant's role in anemia is well-established, its co-occurrence with a platelet disorder underscores the diagnostic challenges in interpreting multiple overlapping phenotypes. The somatic mutations identified in DNMT3A and ASXL1 added another layer of complexity.

These mutations are hallmarks of clonal hematopoiesis, frequently observed in conditions such as Clonal Hematopoiesis of Indeterminate Potential (CHIP) and Clonal Cytopenia of Undetermined Significance (CCUS) [12-15]. While they are associated with an increased risk of progression to MDS or acute myeloid leukemia (AML), the bone marrow findings in this patient, characterized by grade 1 cytopenia without significant dysplasia or clonal expansion, suggested an early-stage condition. Importantly, these mutations alone could not fully explain the severity of the patient's thrombocytopenia and anemia, emphasizing the need to integrate both germline and somatic findings for comprehensive diagnosis and risk assessment. The germline MUTYH deletion (ENST00000456914.7.1353_1355del) added a significant dimension to the patient's risk profile. MUTYH plays a critical role in base excision repair, and its dysfunction predisposes individuals to oxidative DNA damage, promoting clonal evolution and increasing the risk of hematologic malignancies [16]. In this patient, the MUTYH mutation likely predisposed to CHIP/CCUS and increased the likelihood of progression to higher-grade MDS. This finding underscores the importance of long-term monitoring and personalized surveillance strategies in patients with germline mutations linked to hematologic malignancies.

The advanced molecular diagnostics employed in this case facilitated a nuanced understanding of the patient's cytopenias. Integrating somatic and germline findings not only clarified the etiologies but also informed the risk stratification and management plan. For this patient, a personalized follow-up strategy includes regular monitoring of blood counts, metabolic screening to account for potential CD36-related complications, and surveillance for progression to MDS or AML. These findings emphasize the necessity of a multifaceted approach to hematologic disorders, combining clinical presentation, genetic analysis, and long-term follow-up. This case also challenges the limitations of traditional single-gene inheritance models and highlights the evolving role of precision medicine in addressing complex genetic disorders. The semi-dominant hypothesis proposed for the CD36 mutation, the independent contributions of the HBB variant, and the interplay between germline and somatic mutations demonstrate the intricate relationships between genetic variants and phenotypic expression.

The novel insights presented here expand the current understanding of CD36-related disorders and highlight the importance of ongoing research into the functional and clinical implications of heterozygous variants in genes traditionally associated with recessive inheritance. Future studies should explore the broader implications of CD36 mutations in heterozygous carriers and investigate their contributions to platelet and metabolic phenotypes across diverse populations. In conclusion, this case illustrates the value of integrating comprehensive genetic analyses into routine clinical practice. By unraveling the interplay between somatic and germline mutations and exploring novel inheritance paradigms, this report advances the understanding of the genetic basis of cytopenias and underscores the transformative potential of precision medicine in improving diagnostic and therapeutic outcomes.

Acknowledgement

We extend our heartfelt appreciation to our colleague at the University of Selcuk, Faculty of Medicine, Department of Genetics, Konya, Turkey, for their valuable contributions to this study. We also acknowledge the collaborative efforts of various departments within our institution that played a crucial role in the patient's diagnosis. Lastly, we express our deep gratitude to the patient for their participation and endurance.

Conflict of Interest

All the authors confirm that they have no conflict of interest.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2024.60.009384

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