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Beta-lactam antibiotic-induced thrombocytopenia: MYH9 & TUBB1 genes

Two different genetic thrombocyte defects in mother and her son

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Abstract
Aim: Macrothrombocytopenia is a congenital autosomal-dominant blood disorder characterized by increased platelet size and a decreased number of circulating platelets. In this study, it was aimed to show the MYH9 and TUBB1 gene changes, which are the genes associated with the disease, in a patient with thrombocytopenia receiving beta-lactam antibiotic therapy.
Material and Methods: In this study, coagulation parameters and platelet aggregation tests were performed after ingestion of a beta-lactam antibiotic in an 8-year-old boy with thrombocytopenia, the MYH9 and TUBB1 genes were scanned by PCR and DNA sequencing, and the results were subsequently analyzed using bioinformatics tools.
Results: We found previously described TUBB1 polymorphisms, p.R307H , p.Q43P, p.T178T and the novel mutation p.K64A in the MYH9 gene in a boy and his mother. Changes in genes important for thrombocytopenia in a boy after taking beta-lactam antibiotics prompted us to study the same genes in the mother, since her mother had macrothrombocytopenia, and we found a new mutation in her mother
Discussion: Determination of gene changes after beta-lactam antibiotic use in bleeding patients is important in terms of helping the clinic in the treatment.

Keywords
Platelets, Macrothrombocytopenia, MYH9

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Introduction

Myosin heavy chain 9 (MYH9)-related platelet disorders belong to the group of inherited thrombocytopenias. The MYH9 gene, located in chromosomal region 22q12-13, encodes a hexameric protein called 453 kDa non-muscle myosin IIA (NMMIIA), which is involved in cell motility, megakaryocyte contraction, and maintenance of cytoplasmic structure. Studies have shown that mutations in the MYH9 gene cause premature release of platelets from the bone marrow, macrothrombocytopenia, and formation of cytoplasmic inclusion bodies in neutrophils. MYH9-related disorders can have many signs and symptoms, including bleeding problems, kidney disease, hearing loss, and cataracts [1-5]. Macrothrombocytopenia is a congenital autosomal dominant blood disorder characterized by increased platelet size and decreased circulating platelet count. Macrothrombocytopenia belongs to the genetically heterogeneous group of rare disorders associated with multiple genes, MYH9 (MIM155100), ACTN1 (MIM615193) GP1A (MIM606672), GP9 (MIM173515), FL1 (MIM193067), FLNA (MIM3000017), ITG2A/ITGB3 (MIM 607759/173470), GATA1 (MIM305371), NBEAL2(MIM614169) and VWF (MIM613160). The most common forms of these disorders, such as the May-Hegglin anomaly and Bernard-Soulier syndrome, are associated with mutations in MYH9, which forms a dominant-negative protein that inhibits the function of the wild-type heavy chain protein [6,7]

The gene associated with microtubules in platelets is TUBB1 (tubulin beta-1). Microtubules are known to be composed of alpha and beta heterodimers. Although αβ-tubulin heterodimers are an important component of the cell surface membrane skeleton, they are responsible for intracellular transport of vesicles, cell morphogenesis, and chromosome segregation during cell division in all eukaryotes [8]. Studies have shown that tubulin heterogeneity is important for tissue-dependent functional differences between microtubules of the different alpha/beta tubulin isoforms. The relationship between the membrane skeleton, cytoskeleton, and actin filaments, as well as the intertwining of microtubules, ensure the maintenance of normal platelet morphology [9-12]. Beta-tubulin 1 (class VI, TUBB1), which is specific for hematopoietic cells, is 90% present in the marginal band of platelets. For this reason, there are very few studies on the functions of tubulin proteins in platelet structure and determination of the protein effect of the changes found. The TUBB1 gene is located at position 20q13.32 on the chromosome and encodes 451 amino acids.

Normal platelet function, including secretion, adhesion, and aggregation, is triggered by the binding of agonists (adenosine diphosphate [ADP], epinephrine) to specific receptors on the platelet membrane. Beta-lactam antibiotics inhibit platelet functions in vitro, such as secretion, adhesion, and also aggregation. β-Lactam antibiotics cause platelet dysfunction with bleeding complications [12] Previous in vitro studies have documented reversible inhibition of agonist-receptor interaction. Platelet function does not normalize immediately after drug treatment, suggesting irreversible inhibition of platelet function. It is not worth mentioning that antibiotics, especially the beta-lactam antibiotics, are widely used nowadays [13,14].

In this study, we identified a novel missense mutation in MYH9 &TUBB1 genes and described a patient with significant inhibition of platelet function in adenosine diphosphate (ADP) under beta-lactam antibiotics.

Material and Methods

Patient history

We report an 8-year-old boy who was admitted to our department seven days after tonsillectomy for evaluation of the underlying defect with bleeding. His own history revealed two different bleeding episodes: one, as mentioned above, and the other, an epistaxis episode 2 years earlier. The common point for these two attacks was the administration of "beta-lactam antibiotics." The values of all factors were within the normal range; platelet count: 234000/ [150000-450000]/µl, Mean platelet volume: (MPV) 11.1/ [7.20-11.1] fl, Procalcitonin (PCT): 0.26/ [0.10-0.41] %). While coagulation parameters were normal, platelet aggregation showed impaired aggregation in the boy ADP during "beta-lactam antibiotic" treatment. After discontinuation of therapy, the platelet aggregation test was repeated and proved to be normal. Family history indicated that her mother always had thrombocytopenia. Further examination of her mother revealed that she had macrothrombocytopenia. Her platelet count was 105000/ [150000-450000]/µl, mean platelet volume was (MPV) 13.9/ [7.20-11.1] fl, procalcitonin (PCT) was 0.15/ [0.10-0.41] %). Although the scenario and clinical evaluation of the child were quite straightforward and obviously unrelated to this possible finding in the mother.

Genotyping Analysis

Written, informed consent for genetic analysis was obtained from the patients. Approval was also obtained from the Ankara University Clinical Research Ethics Committee in order to comply with the ethical rules of the study. DNA was isolated by proteinase K and phenol/chloroform extraction. Following extraction, all exons of the MYH9 gene and TUBB1 gene were screened by polymerase chain reaction (PCR) with the primers. PCR reactions were performed using 25 ng DNA and GoTaq green master mix reagent in a 20 µL PCR volume (Promega, Wisconsin, USA). Primers were designed using an online tool from the Santa Cruz Genome Browser1. Sequences were analyzed with the Finch TV program.

In silico analysis for predicting pathogenicity of mutations

The potential effects of the missense mutations that were identified in the TUB1 and MYH9 genes were analyzed through two pathogenicity assessment tools. PolyPhen-2 (available at: http://genetics.bwh.harvard.edu/pph2/) is a versatile bioinformatics tool that estimates the potential structural and functional consequences of Mutation/Single Nucleotide polymorphisms on amino acid position. It provides the estimation results between the score interval of 0.0 (benign) and 1.0 (potentially damaging). SNAP 2 (available at: https://rostlab.org/services/snap2web/) is a bioinformatics program that analyzes the functional impact of mutations and SNPs on protein and predicts their effects on phenotypic properties through SNAP2 [17]. When the given values are between (–100-0) and (0–100), mutations are scored as neutral and affected, respectively, in the SNAP software. Furthermore, using the "Multiple sequence alignment" option of the detected missense

mutations in the Poly-Phen2 program, a comparison of amino acid sequences affected by the detected mutations was made between different species.

Structural modeling of selected mutants was calculated by the SWISS-MODEL server. It is a standalone software that can generate mutated models of the proteins for the corresponding amino acid substitutions

Results

The TUBB1 gene functions in the building of the marginal band in platelets, a unique cytoskeleton structure that is composed of bundles of circumferential microtubules that support the maintenance of the shape and function of platelets. The 7401 base-pair gene is located on human chromosome 20q13.3 and consists of 4 exons, encoding 451 amino acids. When we investigated the boy and his mother, we showed the previously-described TUBB1 variant c.920 G>A (p.R307H) at exon 4 and described synonymous variant, c.803G>T (p.T178T) in the boy; p.R307H and p.T177T mutations are important for the gene function, we detected p.Q43P polymorphism, which is frequently seen in the TUBB1 gene, in the mother. The human MYH9 gene contains 41 exons spanning about 33,320 bases and is located on chromosome 22q12-13 [2]. Over 80 different MYH9 mutations have been identified in the Human Gene Mutation Database. In the present study, we identified a novel missense mutation and added c.197G>C to the spectrum of MYH9 mutations. The mutation was predicted to be damaging by both SNAP and Poly-Phen2 software. Although the child's scenario and clinical evaluation seem unrelated to this possible finding in the mother, it is important for the study that a novel mutation has been detected in the mother

MYH9 analysis

According to the analysis results of Poly-Phen2, SNAP and Mutation Taster Database Programs, it was determined that 1 missense nucleotide change detected in our study was not pathogenic because their pathogenic scores were close to 0 and "benign" according to the Poly-Phen 2 program. However, the novel p.K64A missense nucleotide change detected in MYH9 gene was determined to be disease-causing in the results of SNAP and Mutation Taster Programs. The mutations detected are shown in detail in Table-1.

In addition, the missense nucleotide change was detected. By using the "Multiple sequence alignment" option in the Poly-Phen2 program, the amino acid sequences affected by the detected nucleotide were compared between different species. As a result of this analysis, it was determined that 1 missense nucleotide change changed the amino acid at the critical point that were conserved among different species throughout the evolutionary process. Swiss model workspace was applied to model the molecular structures of the wildtype and mutant proteins. Figure 1 shows the location of codons affected by MYH9 and the molecular structures of wild-type and mutant proteins. The change of novel p.K64A missense mutation caused a significant conformational change.

TUBB1 analysis

When the results were analyzed using the Poly-Phen2, SNAP, and Mutation Taster Database programs, it was determined that the p.R307H alteration of the 2 missense nucleotide alterations detected in our study was not pathogenic because their pathogenic scores were close to 0 and "benign" according to the Poly-Phen 2 program. However, SNAP determined that the p.Q43P alteration was effective and disease-causing. Using the multiple sequence alignment option in the Poly-Phen2 program, the amino acid sequences affected by the detected mutations were compared between species. The Swiss Model Workspace was used to model the molecular structures of the wild-type and mutant proteins. Figure 2 shows the location of the codons affected by TUBB1 and the molecular structures of the wild-type and mutant proteins. Alteration of the p.Q43P and p.R307H missense nucleotides does not result in a significant conformational change.

Discussion

Antibiotic-associated thrombocytopenia results from platelet destruction or a reduction in the number of megakaryocytes by the immune mechanism. Studies have shown that beta-lactam antibiotics (penicillin, ampicillin, cefazolin, etc.) can stimulate platelet destruction [11,15]. Cazenave et al. found that beta-lactam antibiotics inhibit platelet functions such as secretion, adhesion and also aggregation in vitro. β-Lactam antibiotics cause platelet dysfunction with bleeding complications. Previous in vitro studies documented reversible inhibition of agonist-receptor interaction. Platelet function does not normalize immediately after drug treatment, suggesting irreversible inhibition of platelet function [16,17]. It is not worth mentioning that antibiotics, especially the beta-lactam antibiotics, are widely used nowadays. Here, we describe a boy and his mother with significant inhibition of platelet function in adenosine diphosphate (ADP) under beta-lactam antibiotics. In the case of beta-lactam-induced thrombocytopenia, when we examined MYH9 and TUBB1 genes, two important genes for platelet function, in the patient and his mother, we found important gene alterations for thrombocytopenia.

We found 3 types of previously described TUBB1 polymorphisms: p.R307H, p.Q43P, p.T178T, and the novel mutation p.K64A in the MYH9 gene. Recent studies have shown that these

Table 1. Mutation of the MYH9 &TUBB1 gene in the patient

No	Gene	Nt alteration	Rs Number	Alteration type	Localization	AA position	Clinical significance		
							Poly-Phen2	SNAP	Mutation Taster
1	MYH9	c.197 G>C	Novel	Splice site change	Exon-1 / Globular Head Domain	p. K64A	-	Effect 18	Polymorphism Protein may be affected
2	TUBB1	c.803G>T	-	Synonymous	MAP Domain	p. T178T	Benign	Neutral	Polymorphism
3	TUBB1	c.920G>A	COSV53885866	Missense	MAP Domain	p.R307H	Benign	Neutral	Polymorphism
4	TUBB1	c.130-131AG>CC	COSV53887225-	Missense	GTP Domain	p.Q43P	Probably Damaging	Effect	Polymorphism

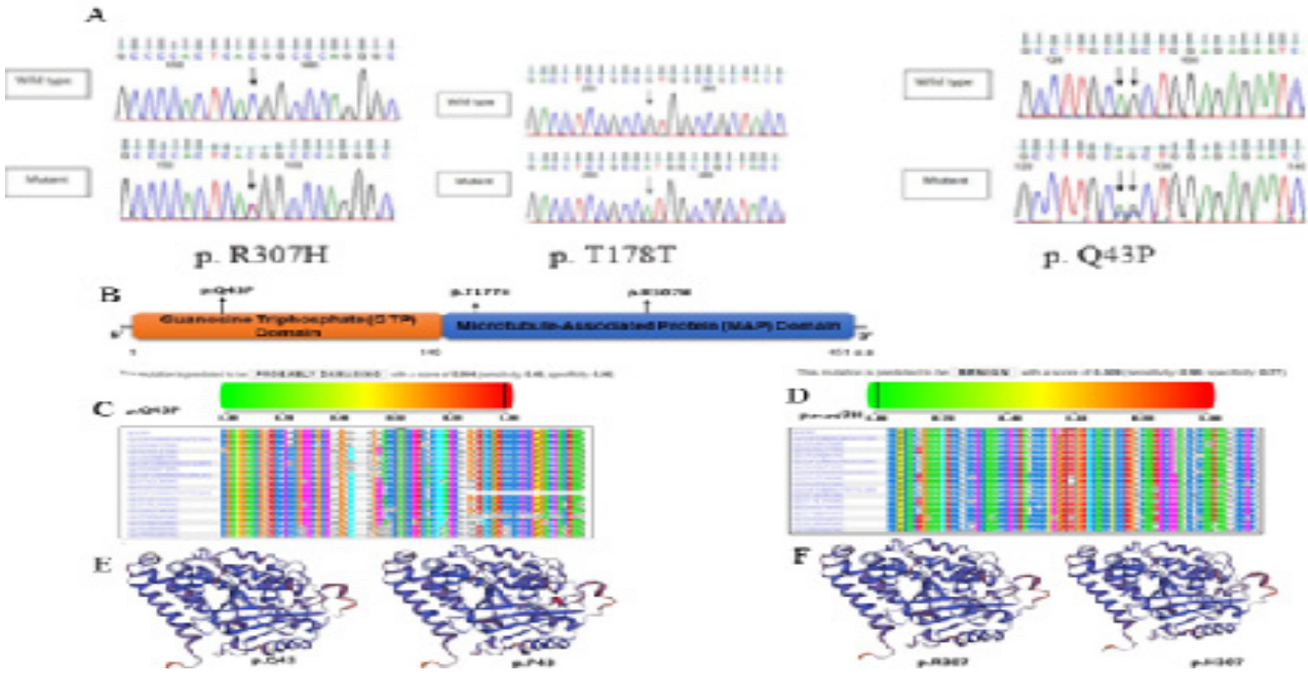

Figure 1. . (A) . Sequencing electropherograms of (A) MYH9 mutant genotype (B) MYH9 normal genotype. Arrows indicate localizations of the mutation and variant. (B) Schematic representation of domain architecture of the MYH9 protein and mutation detected in the patient with thrombocytopenia. Human MYH9 is a polypeptide of 1960 amino acids. Estimation of possible functional effects of mutations in the MYH9 gene with PolyPhen-2 (C) and SNAP (D). Evolutionary conservation analysis of the mutated amino acid in MYH9 gene in the present study. The mutated amino acid was demonstrated. The detected mutant amino acid was evaluated among different species (D). Structure models of the wild-type (E) and mutant MYH9. MYH9 wild-type and K64A mutation (F).

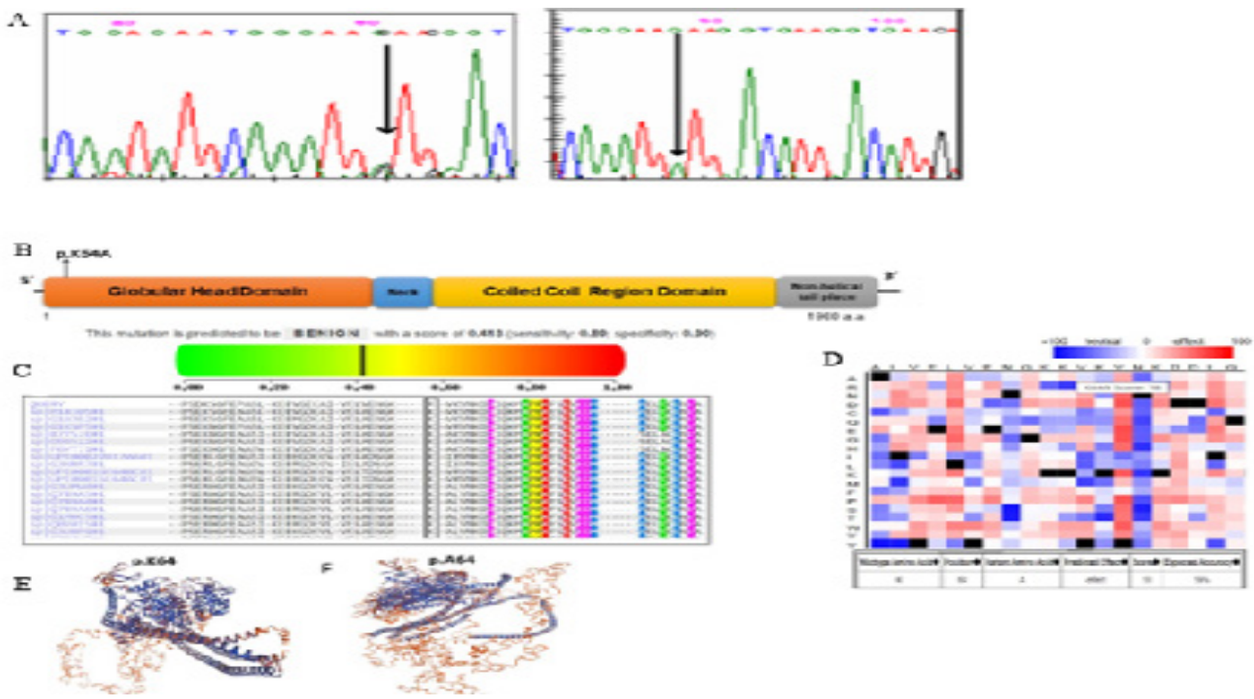

Figure 2. (A) Sequencing revealed heterozygous TUBB1 variations in thrombocytopenia patient. (B) Schematic representation of domain architecture of the TUBB1 protein and mutation detected in a patient with thrombocytopenia. Human TUBB1 is a polypeptide of 451 amino acids. Estimation of possible functional effects of mutations in the TUBB1 gene with PolyPhen-2. The evolutionary conservation analysis of the mutated amino acids in the TUBB1 gene in the present study. The mutated amino acids were demonstrated (C and D). The detected mutant amino acids were evaluated among different species. Structure models of the wild-type (E and F) and mutant TUBB1. TUBB1wild-type and P43/H307 mutation

mutations cause worn cytoskeleton formation and affect platelet morphology. In humans, the R307H variant causes defects in microtubule growth and depolymerizes microtubules. Studies have shown that the p.R307H variant may be useful as a biomarker in immune thrombocytopenia because it may affect platelets, resulting in lower platelet turnover. The Q43P polymorphism is known to result in impaired tubulin organization. Heterozygous carriers of P.Q43P have been found to have spherocytic platelets and platelets enlarged in the cytoplasm. In this study, in which we demonstrated that beta-lactam antibiotics inhibit platelet function, including aggregation, we also detected the p.K64A mutation, identified for the first time in the literature, by maternal genetic and clinical analysis.

In conclusion, MYH9 and TUBB1 gene alterations have been defined in beta-lactam-induced thrombocytopenia, and clinical trials are aimed at investigating the underlying defects in patients with bleeding.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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