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**TBXA2R gene variants associated with bleeding**

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**Abstract**

Platelet activity is regulated by a number of surface expressed G protein-coupled receptors (GPCRs) including the a isoform of the thromboxane receptor (TPα receptor). With the advance of genomic technologies, there has been a substantial increase in the identification of naturally occurring rare GPCR variants including in the TBXA2R gene, which encodes the TPα receptor. The study of patients with naturally occurring variants within TBXA2R associated with bleeding and abnormal TPα receptor function has provided a powerful insight in defining the critical role of TPα in thrombus formation. This review will highlight how the identification of these function-disrupting variants of the platelet TPα has contributed important structure-function information about these GPCRs. Further we discuss the potential implications these findings have for understanding the molecular basis of mild platelet based bleeding disorders.

**Keywords**

Bleeding, GPCR, structure–function, platelets, rare variants, thromboxane receptor TPα

**History**

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**Abbreviations**

GPCR, G protein-coupled receptor; ECL, Extracellular loop; ICL, Intracellular loop; TMD, Transmembrane domain

Platelet activity is regulated by a number of cell surface receptors, including the G protein-coupled a isoform of the thromboxane receptor (TPα receptor). The human TPα receptor was the first human eicosanoid receptor cloned and is a typical Class A rhodopsin-like G protein-coupled receptor (GPCR) (1) with sequence variants in TBXA2R, which encodes the TPα receptor implicated in asthma, atopic dermatitis, and, of particular relevance to this review, an autosomal dominant bleeding disorder (2).

The ligand for the TPα receptor, thromboxane A2 (TXA2) is a product of the oxidative metabolism of arachidonic acid generated by the platelet, thereby acting in an autocrine manner to stimulate TPα receptors (3). Resulting stimulation of Gαq11 and G12/13 heterotrimeric G proteins activates downstream signaling proteins including phospholipase C and RhoA to promote platelet activation. The TXA2 pathway is the target for the most widely prescribed antiplatelet drug aspirin, which irreversibly inhibits cyclooxygenase enzymes (COX-1) reducing platelet TXA2 generation and TPα receptor stimulation. Despite the efficacy of aspirin, there is still interest in developing direct TPα receptor antagonists in order to preserve the beneficial effects of other prostanooids (such as gastric mucosal protection) that are lost upon global COX inhibition (3,4).

One powerful approach to understanding pathophysiological disease mechanisms is the study of patients with bleeding disorders. For example, analysis of pedigrees with the severe platelet function disorder Glanzmann thrombasthenia assisted discovery of the key platelet integrin αIIbβ3 (5). As with most GPCRs, some insights into TPα receptor biology have emerged from the large number of mutagenesis studies undertaken in order to further understand structure–function relationships of the TPα receptor (3). However, studying the direct impact of in vitro mutagenesis on anucleate platelet function in vivo is not possible experimentally. The study of patients with naturally occurring variants within TBXA2R associated with bleeding and abnormal TPα receptor function has provided a powerful alternative defining the critical role of TPα in thrombus formation.

Thromboxane receptor deficiency (MIM #614009) associated with loss of function TBXA2R variants is an autosomal recessive or dominant disorder and has been identified in multiple pedigrees in which some individuals have mild mucocutaneous bleeding symptoms (2). To date, one quantitative defect causing reduced TPα receptor expression (2) and four qualitative defects caused by the TPα receptor amino acid substitutions have been reported (6–9; see Table I and Figure 1).

A nucleotide variation which caused loss of TPα receptor expression was first described in a patient with a history of mucocutaneous bleeding (2). Sequence analysis of TBXA2R in the patient and her father revealed heterozygosity for a single nucleotide duplication at c.167 (c.167dupG in NM_001060.5) resulting in a frame shift from amino acid 58. Corresponding cell lines studies showed that this nucleotide variation significantly reduced receptor expression.

The first reported qualitative defect in the TPα receptor was caused by a missense TBXA2R variant predicting the p.Arg60Leu substitution in the TPα receptor at the start of the first intracellular loop (Figure 1) (9). This variant was first described in a patient with a history of postsurgical bleeding (9), and has since been described in a further pedigree with a history of mild bleeding (10). Platelets from affected individuals show absent or reduced aggregation to the synthetic TXA2 analog U46619. In Arg60Leu homozygous patients, this defect in aggregation was...
accompanied by a reduction in downstream TXA₂-induced calcium signaling pathways. Interestingly, heterozygous Arg60Leu patients also showed reduced TPα-stimulated platelet aggregation but apparently normal calcium mobilization, suggesting an additional pro-aggregatory effect of TPα receptor activation independent of calcium signaling. The Arg60Leu TPα receptor variant has attenuated receptor responses but comparable ligand-binding affinities and receptor surface expression when compared to wild type (WT) receptor (11). Molecular modeling indicates that Arg60 interacts via hydrogen bonds with Met126 and Arg130 in transmembrane domain (TM)3 and that this interaction is lost when the Arg is substituted for Leu (11). Arg130 is part of the highly conserved D/ERY motif (Figure 1) critical for TPα receptor activation (12). Therefore, in line with previous mutagenesis studies of the ERY motif (12), the Arg60Leu substituted TPα receptor is predicted to be unable to undergo the conformational changes required to promote efficient G protein coupling.

The genotyping and phenotyping of platelets (GAPP) consortium has identified and characterized a series of rare variants in a number of platelet GPCR genes (13,14) including TBXA2R (Table I (6–8)). GAPP developed an approach for the rapid identification and characterization of rare genetic variations causing defects within platelet proteins (13). Identification and subsequent characterization of these mutations have significantly enhanced our understanding of structure–function relationships at the TPα receptor.

One example was identified in a patient with a history of bruising and prolonged epistaxes since infancy (6). TPα receptor-stimulated platelet activity was reduced in the patient whereas other platelet receptor responses were similar to responses in healthy controls. Sequencing of TBXA2R showed a heterozygous c.190G> A variant predicting an Asp304Asn substitution within a highly conserved NPXXY motif in TMD7 (Figure 1). The reduction in TXA2-mediated platelet activation in the patient was due

<table>
<thead>
<tr>
<th>Description</th>
<th>Variation in coding DNA</th>
<th>Inheritance</th>
<th>Region</th>
<th>Defect</th>
<th>Platelet TP receptor phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insertion variant causing frameshift</td>
<td>c.167dupG</td>
<td>Heterozygous</td>
<td>Reduced receptor expression</td>
<td>Small and transient platelet aggregation in response to U46619 (2.5 μM) with marked impairment at higher concentration of U46619 (10 μM).</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>R60L</td>
<td>c.179G&gt; T</td>
<td>Homozygous or heterozygous</td>
<td>Reduced receptor coupling to Gq</td>
<td>Absence of platelet response to 9,11-epithio-1,12-methano-TXA₂ (2 μM).</td>
<td>(9)</td>
<td></td>
</tr>
<tr>
<td>D304N</td>
<td>c.190G&gt; A</td>
<td>Heterozygous</td>
<td>Reduced ligand binding</td>
<td>Absence of platelet aggregation in response to 0.5 mM AA with reduced level of aggregation to higher AA (1 mM and 1.5 mM) concentrations.</td>
<td>(6)</td>
<td></td>
</tr>
<tr>
<td>W29C</td>
<td>c.87G&gt; C</td>
<td>Heterozygous</td>
<td>Reduced surface expression</td>
<td>Platelet aggregation in response to AA (1.5 and 2 mM) markedly reduced.</td>
<td>(7)</td>
<td></td>
</tr>
<tr>
<td>N42S</td>
<td>c.125A&gt; G</td>
<td>Heterozygous</td>
<td>Reduced surface expression</td>
<td>Platelet aggregation and secretion to AA (1 and 1.5 mM) absent.</td>
<td>(8)</td>
<td></td>
</tr>
</tbody>
</table>

The numbering used to describe coding region variants relates to the Ref Seq transcript NM_001060.5. ICL: intracellular loop. TMD: transmembrane domain.

Figure 1. Thromboxane (TP-α) receptor snake plot. Sites of naturally occurring variants found in patients with a bleeding history are highlighted in green. Key amino acid regulatory motifs are highlighted in yellow (specifically RXR ER retention motif; D/NPXY motif, E/DRY motif).
to compromised ligand binding in the Asp304Asn substituted TPα receptor. The NPXXY motif is postulated to weakly stabilize α purinergic receptor in which co-in receptor substitutions presented with mild 2011 receptor variants are insufficient receptor to the receptor 7 variants predicting receptor var- in vivo receptor is unclear. The second TMD1 variant, predicting an Asn42Ser substitu- tion, was identified in a patient with significant postoperative and mucocutaneous bleeding (8). As with Trp29Cys, this variant resulted in reduced TPα receptor surface expression and function with the receptor retained intracellularly, in the trans golgi network (TGN)/ER compartment. Asn42, the most con- served residue in class A GPCRs, is therefore required for correct processing and transport of the TPα receptor to the cell surface. One important observation from this case was that the variant predicting the Asn42Ser substitution was present as a heterozy- gous trait, indicating that platelets are expected to express both variant and WT TPα receptor. Despite this there was a profound ligand-binding in a patient with a bleeding diathesis. Blood. 2010;115:363– 736(e1160800–4).

References


