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Abstract Topic: - Molecular effects of genetic variation

Abstract Title: - Genomic and functional characterization of a rare platelet disorder.

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Aims: - To characterize the rare bleeding disorder in a patient family at laboratory and molecular genetic level.

Methods: - The study was approved by the IEC. Patient provided written informed consent. Peripheral blood of proband and family was collected in sodium citrate and EDTA.

Laboratory Investigations: Platelet count, morphology, PT, APTT, TT, Fibrinogen, vWF-Ag, clotting factor assay and FXIII were performed. Platelet aggregometry was performed using ADP (6µM), Collagen (4µg/ml), Arachidonic Acid (0.5mM), Ristocetin (1.25mg/ml), and TRAP–6 (5mM). ATP secretion assay was performed using ADP (6µM), Collagen (4µg/ml) and TRAP–6 (5mM). Expression of GPIX (CD42a), GPIb (CD42b), GPIIbIIIa (CD41a) and GPIV (CD36), CD34 was assessed by flow Cytometry. Platelet activation status was studied using ADP (6µM), TRAP–6 (5mM) and assessment of CD62P, CD63 expression by flow Cytometry. Whole exome sequencing by NGS was performed to detect likely pathogenic mutation related to platelet function disorder. Genetic study of family by Sanger sequencing followed by bioinformatics analysis of the novel missense variant was performed.

Results: - Patient had normal platelet count and ISTH-BAT score of 7. PT, APTT, TT, Fibrinogen, vWF:Ag, factor assays and FXIII were normal. Platelet aggregation with collagen (4µg/ml) was reduced. Whereas, with ADP (6µM), Arachidonic Acid (0.5mM), Ristocetin (1.25mg/ml), and TRAP–6 (5mM) was normal. ATP secretion with ADP (6µM), Collagen (4µg/ml) and TRAP–6 (5mM) was reduced. Expression of GPIX, GPIb, GPIIbIIIa and GPIV were normal by flow cytometry. Low expression of stimulated CD62P (α-granules) and increased platelet CD34 expression was observed in index case and father. The father had similar bleeding history in childhood. All the family members had normal platelet count and morphology. NGS revealed a novel heterozygous missense variant in Exon 10 of the GFIB gene (c.712C<T), that resulted in p.His238Tyr substitution. The father was heterozygous for the variant. Bioinformatics analysis predicted to affect the protein function. Using the clinical exome data, Sanger sequencing was performed in rest of the family members.

Conclusions: - This case was a part of a larger study on rare, unclassified platelet disorders. We identified several disease causing variants in rarely reported genes in patients with platelet disorders. This, and such rare cases indicate the importance of detailed investigations in patients presenting with mild to moderate bleeding diathesis.

Keywords: - Patient had normal platelet count and ISTH-BAT score of 7. PT, APTT, TT, Fibrinogen, vWF:Ag, factor assays and FXIII were normal. Platelet aggregation with collagen (4µg/ml) was reduced. Whereas, with ADP (6µM), Arachidonic Acid (0.5mM), Ristocetin (1.25mg/ml), and TRAP–6 (5mM) was normal. ATP secretion with ADP (6µM), Collagen (4µg/ml) and TRAP–6 (5mM) was reduced. Expression of GPIX, GPIb, GPIIbIIIa and GPIV were normal by flow cytometry. Low expression of stimulated CD62P (α-granules) and increased platelet CD34 expression was observed in index case and father. The father had similar bleeding history in childhood. All the family members had normal platelet count and morphology. NGS revealed a novel heterozygous missense variant in Exon 10 of the GFIB gene (c.712C<T), that resulted in p.His238Tyr substitution. The father was heterozygous for the variant. Bioinformatics analysis predicted to affect the protein function. Using the clinical exome data, Sanger sequencing was performed in rest of the family members.