Annals of Case Reports
Boeckelmann D, et al. Ann Case Rep: 8: 1426
www.doi.org/10.29011/2574-7754.101426
www.gavinpublishers.com

Case Report

Platelet Storage Pool Disease in a Family with Thrombocytopenia and a Pathogenic Variant in the α-Actinin Encoding Gene ACTN1

Doris Boeckelmann[§1], Jessica Klima-Frysch[§2], Hristo Georgiev[1], Rebekka Wiedenhöfer[1], Hannah Glonnegger[1], Barbara Zieger[1*]

[1]Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Faculty of Medicine, Medical Center-University of Freiburg, Freiburg im Breisgau, Germany

[2]Division of Pediatric Surgery, Department of General and Visceral Surgery, Faculty of Medicine, Medical Center-University of Freiburg, Freiburg im Breisgau, Germany

§ shared authorship

***Corresponding author**: Barbara Zieger, Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Faculty of Medicine, Medical Center, University of Freiburg, Freiburg im Breisgau, Germany

Citation: Boeckelmann D, Klima-Frysch J, Georgiev H, Wiedenhofer R, Glonnegger H, et al (2023) Platelet Storage Pool Disease in a Family with Thrombocytopenia and a Pathogenic Variant in the α-Actinin Encoding Gene ACTN1. Ann Case Report. 8: 1426. DOI:10.29011/2574-7754.101426

Received: 24 August 2023, **Accepted:** 29 August 2023, **Published:** 31 August 2023

Abstract

ACTN1-related thrombocytopenia (ACTN1-RT) is an inherited platelet disorder, normally associated with moderate/mild macrothrombocytopenia and a mild bleeding diathesis. Whereas immunofluorescence studies describing the cytoskeleton organization have been reported several times, expression analyses of platelet CD62-P and CD63 using flow cytometry have rarely been performed. We report on familial thrombocytopenia, i.e. four patients – mother and three daughters with moderate/mild thrombocytopenia who were identified as having a pathogenic variant (p.Arg46Trp) in ACTN1. Interestingly, in two of the four patients, flow cytometry analysis showed a profound decrease in surface expression of platelet activation markers CD62-P and CD63. The other two patients showed a mild reduction with different concentrations of thrombin and reached normal exposure after activation with 1 U/mL thrombin. Reduced expression of CD62-P and CD63 is hinting to an α- and δ-storage pool disease (α-/δ-SPD), respectively. Whereas a CD62-P reduction has been previously reported once, this – to our knowledge – is the first report of a CD63 reduction in association with an ACTN1-RT. Patients with platelet SPD are at higher risk for bleeding problems, especially after trauma or surgery. In summary, comprehensive investigation, including molecular genetic and platelet function analysis is important to classify the individual platelet defect. This may help to prevent unnecessary bleeding problems and to optimize perioperative management.

Keywords: Inherited Thrombocytopenia; Storage Pool Disease; ACTN1

Introduction

IInherited thrombocytopenia (IT) is a genetically heterogeneous group of syndromic and non-syndromic bleeding disorders that coincide with low platelet count and/or impaired platelet function. Of the genes identified so far, autosomal dominant pathogenic or likely pathogenic variants in ACTN1 are associated with a mild non-syndromic form of IT referred to as ACTN1- related thrombocytopenia (ACTN1-RT) or bleeding disorder platelet-type 15 (OMIM #615193). ACTN1 is expressed in megakaryocytes and platelets, and encodes for two isoforms of α-actinin. α-actinin contains an actin binding region comprising two calponin homology (CH) domains at the N-terminus, four spectrin repeats and a calmodulin-like motif at the c-terminus

(CaM) [1,2]. Binding regions of the α-actin molecule dimerize into rod-like structures that allow for cross-linking of actin filaments into bundles. In cooperation with other cytoskeletal proteins, actin filaments play an important role in platelet morphology [3]. A disruption of actin filament cross-linking appears to disrupt cytoplasmic organization, according to electron microscopy and immunofluorescence studies of affected megakaryocytes [4]. In primary hemostasis, cytoplasmic organization and contraction are essential for exocytosis of secretory vesicles following platelet activation [5]. Patients with ACTN1-RT present with (mild) macrothrombocytopenia as well as a mild bleeding tendency. While spontaneous bleeding is rare; patients may experience epistaxis, gum bleeding, easy bruising or menorrhagia.

Case Presentation

A 14-old-year old girl (IP) and her mother (M) presented to the outpatient clinic of the Department of Pediatrics and Adolescent Medicine in Freiburg with a history of thrombocytopenia of unknown origin. The girl´s history included easy bruising, specifically large hematomas in response to subtle injury, as well as menorrhagia since the age of 10. The menorrhagia had been successfully treated with hormonal contraceptive therapy. Prior operations included a previous tooth extraction that led to post-operative bleeding in the first night after the procedure. Her mother was 47-years old at the time of presentation. Thrombocytopenia of unknown origin was first diagnosed following the birth of her eldest daughter 20 years prior. She also suffered from easy bruising; however, no excessive bleeding occurred during or after the birth of her three children, (one natural birth, two C-sections). It was known that the two sisters of the IP also suffered from mild thrombocytopenia; however, a comprehensive platelet analysis and molecular genetic genotyping was only performed after diagnosis of an ACTN1-RT in the family. The older sister (S1) experienced hypermenorrhea requiring an hourly change of period products, as well as frequent epistaxis and bleeding gums. Epistaxis would last up to 45 minutes and occurred more frequently under stress. In addition, S1 experienced prolonged bleeding after blood draws along with easy bruising. The younger sister (S2) also reported hypermenorrhea requiring a frequent change of period products, especially during the first 2 days of a period averaging 7 days. She also noticed prolonged bleeding and healing time after small injuries, as well as easy bruising. The father was unaffected from thrombocytopenia and enhanced bleeding; however, had suffered a stroke at the age of 57. External thrombophilia diagnostics had identified homocysteinemia. The index patient (IP) showed a low platelet count of 68 $x10^9$; platelet size was not measurable in automatic cell count systems. Platelet count of the mother was slightly reduced (136 $x10^9$), while the mean platelet volume was increased (MPV 14.3 fL, Norm 7-12 fL). Values for the red blood line were normal; however, there was a measureable increase in reticulocytes (18 promille, norm 3-13 promille) at the date of investigation. The father showed normal platelet count (209 $x10^9$) and mean platelet volume (10.5 fL). Platelet counts for the sisters of the IP (S1 and S2) were 84 $x10^9$ and 106 $x10^9$, respectively. Platelet size was not measurable in automatic cell count systems. Platelet function analysis was performed using light transmission aggregometry (LTA) and flow cytometry analysis of the platelets. The IP showed impaired aggregation in response to epinephrine and low dose ADP. In S2, a mildly impaired aggregation in response to low dose ADP was observed, whereas the mother and S1 reached normal values with different agonists. The father only showed a reduction after stimulation with a low concentration of epinephrine. Ristocetin-induced platelet agglutination was unaffected in all family members (Table 1). Remarkable, preparation of platelet rich plasma reached only 68.000/µl for the index patient. LTA for the mother, S1, and S2 were performed with 185.000 platelets /µL, 103.000/µL, and 162.000/µL, respectively.

	Max. Aggregation[%]				
Agonist	IP	Mother	S1	S2	Father
Collagen (2µg/mL)	76	91	95	88	95
Ristocetin (1.2mg/mL)	86	93	87	91	92
ADP (4µmol/L)	**24**	94	92	60	77
ADP (10µmol/L)	70	n.d.	n.d.	89	n.d.
Epinephrine (8µmol/L)	**0**	91	91	90	**39**
Epinephrine (16µmol/L)	**0**	n.d.	n.d.	n.d.	72

Table 1: Platelet aggregometry analyses. Data are presented as maximal aggregation/agglutination in % compared to normal control levels (>70%). Reduced values are shown in bold.

Platelet flow cytometry (FC) analyses were performed for all thrombocytopenic family members. After activation with thrombin in different concentrations (0.1 – 1 U/mL), the platelets of IP and S2 showed a markedly reduced exposure of the platelet P-selectin (CD62) and CD63 compared to healthy controls. Platelets of the mother and S2 showed reduced exposure of CD62 and CD63 after activation with 0.1 U/mL thrombin; however, exposure after stimulation with 1 U/mL thrombin reached normal values (Figure 1).

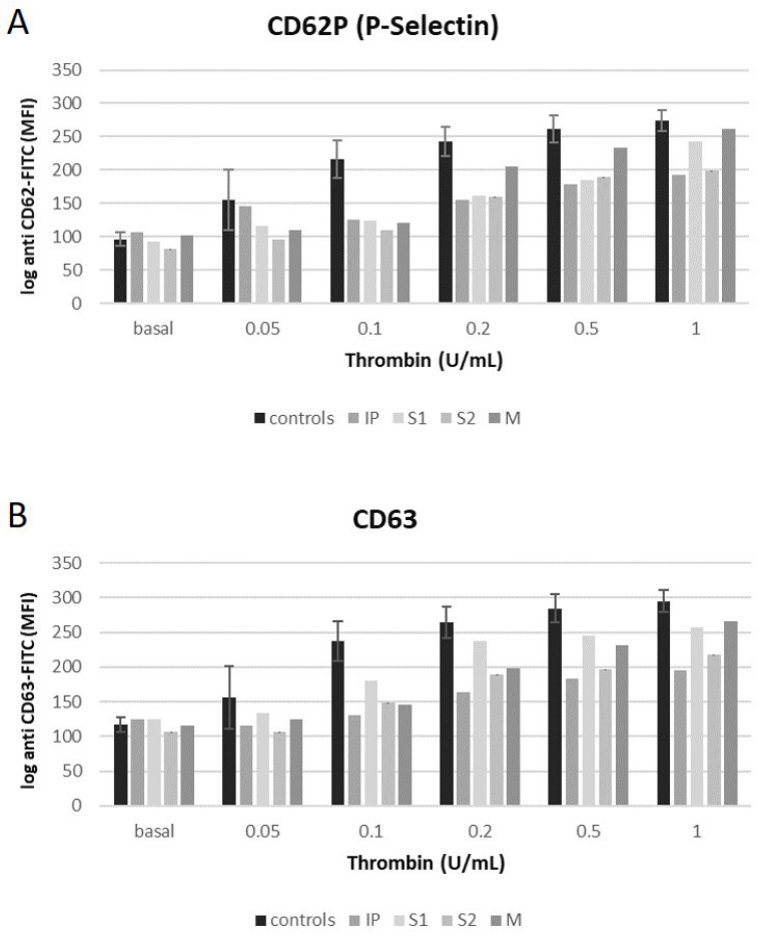

Figure 1: Platelet surface exposure of CD62-P (A) and CD63 (B) stimulated with thrombin (concentrations: 0, 0.05, 0.1, 0.2, 0.5, 1 U/mL) for all thrombocytopenic family members compared to healthy controls. Data are expressed as logarithmic arbitrary units (logAU).

Next generation sequencing of a 95-gene panel was performed as TRIO-analysis for the IP and her parents using a custom designed hybridization based enrichment kit (Flex, Illumina) followed by sequencing on a MiSeq (Illumina). A list of all genes included in the panel has been previously described [6]. The average sequencing depth over all genes for the investigated family members was ≥ 98% for a coverage of x100. The IP and her mother, both affected from thrombocytopenia, shared a heterozygous non-synonymous coding single nucleotide variant 116 (rs747559032) in exon 2 of the ACTN1-gene (NM_001130004.1(ACTN1):c.136C>T, p.Arg46Trp) confirmed by Sanger sequencing. In the population database gnomAD (v.2.1.1) the variant is observed in one allele out of 249952 alleles studied (MAF 0.0004%). In ClinVar the variant is listed with pathogenic and likely pathogenic classifications (Accession: VCV000626995.6). The ISTH-SSC Subcommittee on Genomics in Thrombosis and Hemostasis classified the variant as likely pathogenic (last update: May 21, 2022). The p.R46W alteration is predicted to be deleterious by in silico analysis (CADD score 35 (v1.3)). Genotyping using direct sequencing identified the heterozygous ACTN1 variant also in both affected sisters of the IP. The specific ACTN1 variant identified in the thrombocytopenic family members results in an aminco acid substitution at a highly conserved amino acid position. The p.R46 amino acid is conserved in vertebrate species and down to the fruit fly. The amino acid substitution (p.Arg46Trp) caused by the variant has been described as autosomal dominant pathogenic variant associated with ACTN1-RT. The c.136C>T (p.R46W) alteration co-segregated with disease in several unrelated families with inherited thrombocytopenia and platelet macrocytosis [7-10]. In addition, an alteration affecting the same codon, p.R46Q, were observed in two additional unrelated families with macrothrombocytopenia [4,11]. R46W is located in the N-terminal actin binding (AB) domain. Faleschini et al. were able to demonstrate using protein mapping that Arg46 is positioned at the end of helix 1 and across from Asp217, with

which it forms a salt bridge. This is assumed to provide structural stability and to maintain the orientation of both CH domains at the N-terminus of α-actinin 1, which are responsible for actin binding. The described amino acid substitution significantly disrupts this stability and impairs actin filament organization [10]. Functional analysis demonstrated that the p.R46W alteration prevents filament formation and co-localizing of ACTN1 with actin. Bottega et al. performed immunoflourescence assays in human fibroblasts transfected with wild-type constructs and mutant R46W constructs [7]. They observed that in wild-type cells, the cytoskeleton was organized in filaments with ACTN1 co-localizing with actin; however, in cells expressing R46W, ACTN1 was distributed uniformly within the cytoplasm and was no longer organized in filaments. Murphy et al. showed increased binding of Arg46Gln actinin-1 to actin filaments; resulting in more stable actin filaments, impairing stretching and leading to shorter and thicker fibers in transfected CHO cells [12]. Boutroux et al. described four families with ACTN1 variants (one of them with R46W) and performed platelet flow cytometry for the exposure of activation markers CD62-P and CD63; however, they did not perform this analysis for the family with the R46W variant. For patients with R738Q and R738W ACTN1 variants they reported normal exposure of activation markers, for a patient with D20N ACTN1 variant they described a reduced CD62-P exposure [9].

Discussion

The association of an ACTN1-RT and a functional platelet defect has rarely been reported. Especially the association of an ACTN1-RT with a δ-storage pool disease has not been described thus far in the literature. In combination, however, it seems likely that the disruption of actin filament organization within the platelet cytoplasma leads to impaired secretion of granular content, which depends on cystoskeletal contraction [5]. After platelet activation, platelet granules fuse with the plasma membrane to secrete their contents via exocytosis. Abnormalities in granule synthesis, storage or secretion can lead to impairment of primary hemostasis. Non-syndromic phenotypes of thrombocytopathia are often only moderate; however, in the event of significant trauma or surgery, especially in mucosal areas, significant bleeding can occur [13]. Therefore, for patients the knowledge of the molecular genetic defect is important to understand the phenotype, possible therapy and outcome of their platelet disorder. A precise diagnosis may prevent bleeding problems and inappropriate treatment. Before undergoing major surgery, patients with an ACTN1-RT should receive a platelet function analysis including the analysis of granule secretion after activation. Patients with a platelet secretion defect have a greater risk of bleeding complications during surgery.

Ethic statement: This study was approved by Albert-Ludwigs-University Freiburg's institutional review board (EK584/17 and EK222/20). All procedures were conducted in accordance with the approved guidelines and informed consent of all research participants.

Acknowledgement: We would like to thank Anja Kahle for excellent technical assistance. Barbara Zieger of the authors of this publication is a member of the European Reference Network on Rare Haematological Diseases (ERN-EuroBloodNet) - Project ID N° 101085717.

Funding: The research of BZ was partially funded by CSL-Behring (ZVT No. ZVS-2019092402).

Conflict of interest: All authors declare having no conflict of interest related to the submitted work. All authors have contributed to the writing and approval of the manuscript.

References

1. Otey CA, and Carpen O (2004) Alpha-actinin revisited: a fresh look at an old player. Cell Motil Cytoskeleton, 58: 104-11.
2. Sjoblom, BA, Salmazo, and Djinovic-Carugo K (2008) Alpha-actinin structure and regulation. Cell Mol Life Sci, 65: 2688-701.
3. O'Sullivan, L.R, Cahill MR, and Young PW (2021) The Importance of Alpha-Actinin Proteins in Platelet Formation and Function, and Their Causative Role in Congenital Macrothrombocytopenia. Int J Mol Sci, 22.
4. Gueguen, P, Rouault K, Chen JM, Raguenes O, Fichou Y, et al (2013) A missense mutation in the alpha-actinin 1 gene (ACTN1) is the cause of autosomal dominant macrothrombocytopenia in a large French family. PLoS One, 2013. 8: e74728.
5. Rendu, F. and Brohard-Bohn B (2001) The platelet release reaction: granules' constituents, secretion and functions. Platelets, 12: 261-73.
6. Boeckelmann, D, Wolter M, Neubauer K, Sobotta F, Lenz A, et al (2021) Hermansky-Pudlak Syndrome: Identification of Novel Variants in the Genes HPS3, HPS5, and DTNBP1 (HPS-7). Front Pharmacol, 12: 786937.
7. Bottega, R, Marconi C, Falescini M, Baj G, Cagioni C, et al (2015) ACTN1-related thrombocytopenia: identification of novel families for phenotypic characterization. Blood, 125: 869-72.
8. Vincenot, A, Saultier P, Kunishima S, Poggi M, Roussel A, et al (2019) Novel ACTN1 variants in cases of thrombocytopenia. Hum Mutat, 40: 2258-2269.
9. Boutroux, H, David B, Gueguen P, Frange P, Vincenot A, et al (2017) ACTN1-related Macrothrombocytopenia: A Novel Entity in the Progressing Field of Pediatric Thrombocytopenia. J Pediatr Hematol Oncol, 39: e515-e518.

10. Faleschini, M, Melazzini F, Marconi C, Giangregorio T, Pippucci T, et al (2018) ACTN1 mutations lead to a benign form of platelet macrocytosis not always associated with thrombocytopenia. Br J Haematol, 183: 276-288.

11. Kunishima, S, Okuno Y, Yoshida K, Shirashi Y, Sanada M, et al (2013) ACTN1 mutations cause congenital macrothrombocytopenia. Am J Hum Genet, 92: 431-8.

12. Murphy, A.C, Lindsay AJ, McCaffey MW, Carugo KD, Young PW (2016) Congenital macrothrombocytopenia-linked mutations in the actin- binding domain of alpha-actinin-1 enhance F-actin association. FEBS Lett, 590: 685-95.

13. Dupuis A, Bordet JC, Eckly A, Gachet C (2020) Platelet delta-Storage Pool Disease: An Update. J Clin Med, 9.