A novel RUNX1 exon 3 - 7 deletion causing a familial platelet disorder

Almazni, Ibrahim Abdullah F; Chudakou, Pavel; Dawson-Meadows, Alison; Downes, Kate; Freson, Kathleen; Mason, Joanne; Page, Paula; Reay, Kim; Myers, Bethan; Morgan, Neil

DOI:
10.1080/09537104.2021.1887470

A novel RUNX1 exon 3 – 7 deletion causing a familial platelet disorder

Journal:	*Platelets*
Manuscript ID	CPLA-2020-0580.R1
Manuscript Type:	Case Report
Date Submitted by the Author:	29-Jan-2021
Complete List of Authors:	Almazni, Ibrahim; University of Birmingham Chudakou, Pavel; Lincoln County Hospital Dawson-Meadows, Alison; Lincoln County Hospital Downes, Kate; East Genomic Laboratory Hub Freson, Kathleen; KU Leuven, Cardiovascular Sciences Mason, Joanne; West Midlands Regional Genetics Laboratory Page, Paula; West Midlands Regional Genetics Laboratory Reay, Kim; West Midlands Regional Genetics Laboratory Myers, Bethan; University Hospitals of Leicester NHS Trust, Haemostasis and Thrombosis Morgan, Neil; University of Birmingham,
Keywords:	RUNX1, thrombocytopenia, platelet disorder, NGS, CNV, bleeding

A novel *RUNX1* exon 3 - 7 deletion causing a familial platelet disorder

Ibrahim Almazni[1], Pavel Chudakou[2], Alison Dawson-Meadows[2], Kate Downes[3], Kathleen Freson[4], Joanne Mason[5], Paula Page[5], Kim Reay[5], Bethan Myers[2,6], Neil V Morgan[1]* on behalf of the UK GAPP Study Group

[1]Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK

[2]Department of Haematology, Lincoln County Hospital, Lincoln, UK

[3]East Genomic Laboratory Hub, Cambridge University Hospitals, Cambridge, UK

[4]Center for Molecular and Vascular Biology, Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium

[5]West Midlands Regional Genetics Laboratory, Birmingham Women's Hospital, Birmingham, UK

[6]Department of Haematology, University Hospitals of Leicester, Leicester, UK

Running title: *RUNX1* deletion and platelet disorder

Corresponding author

* Dr Neil Morgan, Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, Edgbaston, University of Birmingham, Birmingham, B15 2TT, UK; E-mail: N.V.Morgan@bham.ac.uk; Tel +44 (0)121 414 6820.

Word count: Text: 1087

Tables 1, Figures 1

References 17

Keywords: RUNX1, thrombocytopenia, platelet disorder, NGS, CNV, bleeding

Abstract

Familial Platelet Disorder with associated Myeloid Malignancy (FPDMM) is a rare inherited disorder confirmed with the presence of a pathogenic germline *RUNX1* variant and is thought to be heavily underdiagnosed. RUNX1 has also been found to be mutated in up to 10% of adult AML cases and other cell malignancies. We performed targeted next generation sequencing and subsequent MLPA analysis in a kindred with multiple affected individuals with low platelet counts and a bleeding history. We detected a novel heterozygous exon 3 – 7 large deletion in the RUNX1 gene in all affected family members which is predicted to remove all of the Runt-homology DNA-binding domain and a portion of the the Activation domain. Our results show that the combination of targetted NGS and MLPA analysis is an effective way to detect copy number variants (CNVs) which would be missed by conventional sequencing methods. This precise diagnosis offers the possiblility of accurate counselling and clinical management in such pateints whom could go onto develop other cell malignancies.

Introduction

Inherited thrombocytopenias (ITs) comprise a heterogeneous group of disorders with a sustained reduction in platelet count which often manifest as a bleeding diathesis. To date 26 forms of IT have been reported with known disease associations in 30 genes which makes genetic diagnosis challenging[1-3]. A precise genetic diagnosis provides clinical benefits for the patients where some patients with IT have unnecessary procedures and treatments such as splenectomies or mistaken for idiopathic thrombocytopenic purpura (ITP) which may be treated with steroids or immunosuppressive drugs with many side effects. Some of the gene variants in IT patients e.g. *RUNX1* result in patients having a predisposition to haematological malignancies and once a genetic defect is proven the information can be used to monitor the patients' haematological parameters more closely. Familial Platelet Disorder with associated Myeloid Malignancy (FPDMM) (OMIM# 601399) is confirmed with the presence of a pathogenic germline mutation in *RUNX1.* *RUNX1* variants have been reported in approximately 10% of adult AML, which are mainly

acquired but can also be germline and thought to be underdiagnosed[4]. This highlights the need for a definitive genetic diagnosis to aid clinical management of the condition.

Current Next Generation Sequencing (NGS) technologies allow the detection of both point mutations and CNVs with a single platform and workflow[5-7]. In this study we describe and elucidate the genetic basis of a single family with 3 affected individuals who suffer from a bleeding diathesis and low platelet counts with previously unknown genetic diagnosis.

Cases

The family presented here has 3 affected individuals, all with a strong family history of clinical bleeding including epistaxis and easy bruising associated with low platelet counts and recruited to the UK Genotyping and Phenotyping of Platelets (GAPP) study[8] (Figure 1A). The index case (P1) was a 67 year old male when initially recruited to the GAPP study with a 10 year history of a 'probable' hereditary platelet disorder. He had a history of bleeding episodes which included epistaxis, cutaneous bleeding, bleeding from minor wounds, GI bleeding, oral cavity bleeding, bleeding after tooth extraction and muscle hematomas. He bled excessively after a hip replacement. He had a platelet count of 129 x 10^9/L and mean platelet volume (MPV) of 10.2 fL. He had Coronary artery bypass graft surgery aged 69 following which he suffered a thromboembolic stroke.

His relatives P2 and P3 (son and grandson respectively) were also referred for genetic testing to the GAPP study. P2 is a 44 year old man with a clinical history of life-long muco-cutaneous bleeding. He had a platelet count of 83 x 10^9/L and MPV of 9.3 fL. P3 is 22 years old with clinical features of platelet dysfunction. His platelet count at testing was 96 x 10^9/L and MPV of 12.6 fL. (Table 1). To date no evidence of AML or other associated haematological malignancies have been reported in any of these patients. All other haematological parameters were within normal ranges.

The index case P1 was subjected to the ThromboGenomics targeted sequencing platform (TGv3.0)[5] where CNVs are called using an experimental pipeline based on ExomeDepth[9] version

1.1.10. This detected a novel heterozygous CNV Deletion of *RUNX1* from exons 3 – 7 (Figure 1B). The genetic variant was scored with strong evidence of pathogenicity based on the current ACMG guidelines[10] (PM2 - Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium, PVS1 - Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease). To confirm if the *RUNX1* CNV deletion was present in all affected patients, Multiplex ligation-dependent probe amplification (MLPA) analysis was performed which includes all exons of the RUNX1/CEBPA/GATA2/TERC/TERT genes (MRC Holland kit P437-B1), and can detect both deletions and duplications. The index patient P1 and relatives P2 and P3 were all confirmed to have the heterozygous *RUNX1* exon 3 – 7 deletion by MLPA analysis (Figure 1C). The deletion removes 870bp of the mRNA sequence of *RUNX1* which is in frame, but completely removes the DNA/Runt DNA binding domain and part of the activation domain (Figure 1D).

Discussion

This report describes a family with an inherited thrombocytopenia and history of excessive bleeding. FPDMM is confirmed in this family studied with the presence of a pathogenic germline *RUNX1* mutation[11-13]. To date more than 70 FPDMM families have been reported with a 40% lifetime risk of developing MDS and/or AML[14]. While the majority of familial cases reported in the literature with *RUNX1* mutations are point mutations, different extended deletions have also been described[5, 11, 15, 16] (Figure 1D). Here using targeted NGS and subsequent CNV/MLPA analysis, we have detected a novel heterozygous deletion covering exons 3 – 7 of the *RUNX1* gene which is predicted to remove the entire DNA Runt/DNA binding domain. *RUNX1*/*AML1* encodes the DNA binding unit of the heterodimeric core binding factor (CBF) that is a critical regulator of definitive hematopoiesis[17]. RUNX1 is also recurrently mutated gene in sporadic myelodysplastic syndrome and leukaemia. In sporadic AML, mutations in *RUNX1* are usually secondary events, whereas in FPDMM they are initiating events. None of the patients included in this study have developed AML as a result of a secondary somatic event occurring within *RUNX1* to date. It is thought that *RUNX1*

variants causing haploinsufficiency are thought to be associated with a lower incidence of myeloid malignancies when compared to those patients with dominant negative *RUNX1* defects. T~~However t~~he detection of a *RUNX1* variant in such patients with low platelet counts allows the possiblility of accurate counselling and clinical management for both them and extended family members at 50% risk of inheriting the pathogenic variant, whom could go onto develop other cell malignancies.

Acknowledgments The authors would like to thank the family and patients for taking part in the study. The work in the author's laboratories is supported by the British Heart Foundation (PG/13/36/30275; FS/15/18/31317; PG/16/103/32650; FS/18/11/33443; NVM) and the Saudi Arabia Cultural Bureau in London (IA).

Authorship contribution NVM designed the study; BM recruited the patients; IA, PC, AD-M, KD, KR, BM and NVM extracted or generated clinical or experimental data; IA, KD, KF, JM, PP, KR and NVM interpreted the results; KD, and NVM undertook governance of the study; BM and NVM wrote the manuscript; all authors read and approved the final version of the manuscript.

Disclosure of Conflicts of Interest The authors report no conflicts of interest

Supplementary Acknowledgements

The members of the UK Genotyping and Phenotyping of Platelets Study Group are: Neil Morgan, Steve Watson, Gillian Lowe, Paul Harrison, Marie Lordkipanidze (University of Birmingham); Andrew D Mumford and Stuart J Mundell (University of Bristol); Paul Gissen (University College London); Martina E. Daly (University of Sheffield); Will Lester and Justin Clark (Birmingham Women's Hospital); Mike Williams, Jayashree Motwani, Dianne Marshall, Natalie Lawson, Priscilla Nyatanga, Pat Mann, and Julie Kirwan (Birmingham Children's Hospital); Will Lester, Charles Percy, Pam Green, Helen Hupston, Koomaravel Nagapachetty, Elizabeth Dwenger, Ann O Rourke, Martin Pope, Camillia Edmead and April Greenway (University Hospital Birmingham); Michael Makris (Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital); Jeanette Payne (Paediatric Haematology Centre, Sheffield Children's Hospital); Sue Pavord, Richard Gooding and Rashesh Dattani (University Hospitals Leicester); Gerry Dolan Charlotte Grimley, Simone Stokley, Emma Astwood, Karyn Longmuir, Cherry Chang, Merri Foros, Michelle Kightley and Linda Trower (Nottingham University Hospitals); Jecko Thachil (previously Paula Bolton Maggs), Charlie Hay, Gill Pike, Andrew Will, John Grainger, Matt Foulkes, and Mona Fareh

(Central Manchester National Health Service Foundation Trust); Kate Talks, Tina Biss, Patrick Kesteven, John Hanley, Julie Vowles, Lesley Basey, Kevin Knaggs and Michelle Barnes (Newcastle upon Tyne Hospitals NHS Trust); Peter Collins, Rachel Rayment, Raza Alikhan, Ana Guerrero Rebecca Morris, and Dianne Mansell (Cardiff and Vale University Local Health Board); Cheng Hock Toh and Vanessa Martlew (Royal Liverpool University Hospitals); Elaine Murphy and Robin Lachmann (University College London Hospitals NHS Trust); Peter Rose, Oliver Chapman, Anand Lokare, Kathryn Marshall, and Naseem Khan (University Hospitals Coventry and Warwickshire); David Keeling, Nikki Curry and Paul Giangrande (Oxford Radcliffe Hospitals NHS Trust); Steve Austin, David Bevan and Jayanthi Alamelu (Guys' and St. Thomas' NHS Foundation Trust); David Allsup, Andrew Fletcher, Katherine Gladstone, Jeanette Fenwick, Philippa Woods and Darren Camp (Hull and East Yorkshire Hospitals NHS trust, Castle Hill Hospital, Hull); Beki James, Suzie Preston, Collette Spencer, Alexandra Pike and Chung Lai-Wah (Leeds Teaching Hospitals NHS trust); Angela Thomas (Royal Hospital for Sick Children Edinburgh); Bethan Myers (Lincoln County Hospital); Gillian Evans, Kim Elliot, Karen Davies, Charlotte Graham and Miranda Foad, (Kent Canterbury Hospital), Jacqueline Smith (The Dudley Group NHS Foundation Trust). Dr Neil Morgan has authorised authorship on behalf of the UK Genotyping and Phenotyping of Platelets Study Group.

References

1 Almazni I, Stapley R, Morgan NV. Inherited Thrombocytopenia: Update on Genes and Genetic Variants Which may be Associated With Bleeding. *Front Cardiovasc Med*. 2019; 6: 80.

2 Johnson B, Doak R, Allsup D, et al. A comprehensive targeted next-generation sequencing panel for genetic diagnosis of patients with suspected inherited thrombocytopenia. *Res Pract Thromb Haemost*. 2018; 2: 640-52.

3 Johnson B, Fletcher SJ, Morgan NV. Inherited thrombocytopenia: novel insights into megakaryocyte maturation, proplatelet formation and platelet lifespan. *Platelets*. 2016; 27: 519-25.

4 Simon L, Spinella JF, Yao CY, et al. High frequency of germline RUNX1 mutations in patients with RUNX1-mutated AML. *Blood*. 2020; 135: 1882-6.

5 Downes K, Megy K, Duarte D, et al. Diagnostic high-throughput sequencing of 2396 patients with bleeding, thrombotic, and platelet disorders. *Blood*. 2019; 134: 2082-91.

6 Almazni I, Stapley RJ, Khan AO, Morgan NV. A comprehensive bioinformatic analysis of 126 patients with an inherited platelet disorder to identify both sequence and copy number genetic variants. *Hum Mutat*. 2020; 41: 1848-65.

7 Lentaigne C, Freson K, Laffan MA, et al. Inherited platelet disorders: toward DNA-based diagnosis. *Blood*. 2016; 127: 2814-23.

8 Watson SP, Lowe GC, Lordkipanidze M, et al. Genotyping and phenotyping of platelet function disorders. *J Thromb Haemost*. 2013; 11 Suppl 1: 351-63.

9 Plagnol V, Curtis J, Epstein M, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics*. 2012; 28: 2747-54.

10 Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015; 17: 405-24.

11 Morgan NV, Daly ME. Gene of the issue: RUNX1 mutations and inherited bleeding. *Platelets*. 2017; 28: 208-10.

12 Preudhomme C, Renneville A, Bourdon V, et al. High frequency of RUNX1 biallelic alteration in acute myeloid leukemia secondary to familial platelet disorder. *Blood*. 2009; 113: 5583-7.

13 Stockley J, Morgan NV, Bem D, et al. Enrichment of FLI1 and RUNX1 mutations in families with excessive bleeding and platelet dense granule secretion defects. *Blood*. 2013; 122: 4090-3.

14 Liew E, Owen C. Familial myelodysplastic syndromes: a review of the literature. *Haematologica*. 2011; 96: 1536-42.

15 Owen CJ, Toze CL, Koochin A, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. *Blood*. 2008; 112: 4639-45.

16 Latger-Cannard V, Philippe C, Bouquet A, et al. Haematological spectrum and genotype-phenotype correlations in nine unrelated families with RUNX1 mutations from the French network on inherited platelet disorders. *Orphanet J Rare Dis*. 2016; 11: 49.

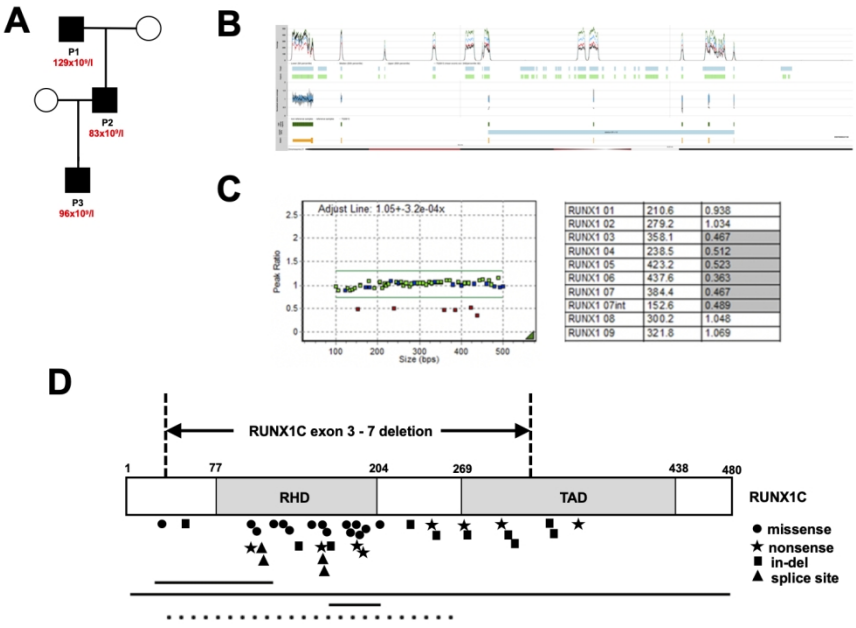
17 Okuda T, Nishimura M, Nakao M, Fujita Y. RUNX1/AML1: a central player in hematopoiesis. *Int J Hematol*. 2001; 74: 252-7.

Table 1. Results of haematological analysis in patients P1-P3 at time of recruitment

Laboratory analysis	**P1**	**P2**	**P3**	**Normal range**
Haemoglobin	14.0 g/dL	14.3	14.2	13.5 - 17.5
MCV	91.8 fl	90.6	93.8	80.0 - 94.0
Haematocrit	40.5 %	40.5	40.7	41.0 - 53
Red cell count	4.41×10^{12}/L	4.47	4.34	3.8 – 6.5
White cell count	5.37×10^{9}/L	6.26	5.08	4.8 - 10.8
Neutrophils	3.71×10^{9} (69.1 %)/L	3.38×10^{9} (54.0%)/L	2.27×10^{9} (44.7%)/L	1.5 - 6.5
Lymphocytes	0.77×10^{9} (14.3%)/L	1.68×10^{9} (26.8%)/L	1.39×10^{9} (27.4%)/L	1.2 - 3.4
Monocytes	0.68×10^{9} (12.7%)/L	0.67×10^{9} (10.7%)/L	0.91×10^{9} (17.9%)/L	0.2 – 0.8
Eosinophils	0.20×10^{9} (3.7%)/L	0.52×10^{9} (8.3%)/L	0.49×10^{9} (9.6%)/L	0 – 0.4
Basophils	0.01×10^{9} (0.2%)/L	0.01×10^{9} (0.2%)/L	0.02×10^{9} (0.4%)/L	0 – 0.1
Platelets	129 $\times10^{9}$/L	83 $\times10^{9}$/L	96×10^{9}/L	147 - 327
Mean platelet volume	10.2 fl	9.3	12.6	7.8 - 12.69
Immature platelet fraction	5.4%	2.3	13.6	1.3 – 10.8
Reticulocytes	67.0 $\times10^{9}$/L (1.52%)	91.6 $\times10^{9}$/L (2.05%)	81.6 $\times10^{9}$/L (1.88%)	20 - 80 (0.2 - 2%)

Figure legend

Figure 1. Identification of a novel germline mutation in RUNX1 in a family with inherited thrombocytopenia (A) Pedigree of family showing affected individuals (shaded) and platelet counts (B) CNV analysis of sequencing data of P1 using ExomeDepth within the ThromboGenomics targeted sequencing platform, detected a novel heterozygous CNV deletion of *RUNX1* spanning from exons 3 – 7. (C) MLPA analysis detects the heterozygous exon 3 -7 deletion in *RUNX1* in all patients (representative data shown from P1 only). MLPA peak ratios for kit P437-B1 including *RUNX1* (red and green) and control (blue) MLPA test probes (left hand panel). Table shows RUNX1 exon number or intron (int), bin size and peak ratios. Red, probes with peak ratios <0.75 indicate a heterozygous deletion (grey shaded) (right hand panel). (D) Schematic showing the location of the novel exon 3 – 7 exons deleted within *RUNX1* which are implicated in the FPD/AML family studied. The Runt-homology DNA-binding domain (RHD) spanning amino acids 77 to 204 and the Activation domain (TAD) spanning from amino acid 269 to 438 is also displayed. Alterations are numbered according to positions in the NM_001754.4 transcript for *RUNX1* (RUNX1c isoform). Positions of different types of published *RUNX1* variants causing FPDMM are also shown where *RUNX1* missense variants are almost exclusively located in the Runt homology DNA-binding domain. Reported gross deletions are shown with solid lines and a gross duplication with dashed line.



594x419mm (72 x 72 DPI)