

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

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

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3 **A novel *RUNX1* exon 3 - 7 deletion causing a familial platelet disorder**
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7 Ibrahim Almazni¹, Pavel Chudakou², Alison Dawson-Meadows², Kate Downes³, Kathleen Freson⁴,
8 Joanne Mason⁵, Paula Page⁵, Kim Reay⁵, Bethan Myers^{2,6}, Neil V Morgan^{1*} on behalf of the UK
9 GAPP Study Group
10
11

12
13
14 ¹Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of
15 Birmingham, Birmingham, UK
16

17
18 ²Department of Haematology, Lincoln County Hospital, Lincoln, UK
19

20 ³East Genomic Laboratory Hub, Cambridge University Hospitals, Cambridge, UK
21

22 ⁴Center for Molecular and Vascular Biology, Department of Cardiovascular Sciences, University of
23 Leuven, Leuven, Belgium
24

25
26 ⁵West Midlands Regional Genetics Laboratory, Birmingham Women's Hospital, Birmingham, UK
27

28 ⁶Department of Haematology, University Hospitals of Leicester, Leicester, UK
29
30
31
32

33 **Running title:** *RUNX1* deletion and platelet disorder
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37 **Corresponding author**
38

39 * Dr Neil Morgan, Institute of Cardiovascular Sciences, College of Medical and Dental
40 Sciences, Edgbaston, University of Birmingham, Birmingham, B15 2TT, UK; E-mail:
41 N.V.Morgan@bham.ac.uk; Tel +44 (0)121 414 6820.
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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 challenging¹⁻³. 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

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2
3 acquired but can also be germline and thought to be underdiagnosed⁴. This highlights the need for
4
5 a definitive genetic diagnosis to aid clinical management of the condition.
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7 Current Next Generation Sequencing (NGS) technologies allow the detection of both point
8
9 mutations and CNVs with a single platform and workflow⁵⁻⁷. In this study we describe and elucidate
10
11 the genetic basis of a single family with 3 affected individuals who suffer from a bleeding diathesis
12
13 and low platelet counts with previously unknown genetic diagnosis.
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15

16 17 18 **Cases**

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20 The family presented here has 3 affected individuals, all with a strong family history of clinical
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22 bleeding including epistaxis and easy bruising associated with low platelet counts and recruited to
23
24 the UK Genotyping and Phenotyping of Platelets (GAPP) study⁸ (Figure 1A). The index case (P1)
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26 was a 67 year old male when initially recruited to the GAPP study with a 10 year history of a
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28 'probable' hereditary platelet disorder. He had a history of bleeding episodes which included
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30 epistaxis, cutaneous bleeding, bleeding from minor wounds, GI bleeding, oral cavity bleeding,
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32 bleeding after tooth extraction and muscle hematomas. He bled excessively after a hip
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34 replacement. He had a platelet count of $129 \times 10^9/L$ and mean platelet volume (MPV) of 10.2 fL.
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36 He had Coronary artery bypass graft surgery aged 69 following which he suffered a
37
38 thromboembolic stroke.
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41 His relatives P2 and P3 (son and grandson respectively) were also referred for genetic testing to
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43 the GAPP study. P2 is a 44 year old man with a clinical history of life-long muco-cutaneous
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45 bleeding. He had a platelet count of $83 \times 10^9/L$ and MPV of 9.3 fL. P3 is 22 years old with clinical
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47 features of platelet dysfunction. His platelet count at testing was $96 \times 10^9/L$ and MPV of 12.6 fL.
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49 (Table 1). To date no evidence of AML or other associated haematological malignancies have
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51 been reported in any of these patients. All other haematological parameters were within normal
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53 ranges.
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58 The index case P1 was subjected to the ThromboGenomics targeted sequencing platform
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60 (TGv3.0)⁵ where CNVs are called using an experimental pipeline based on ExomeDepth⁹ version

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3 1.1.10. This detected a novel heterozygous CNV Deletion of *RUNX1* from exons 3 – 7 (Figure 1B).
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5 The genetic variant was scored with strong evidence of pathogenicity based on the current ACMG
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7 guidelines¹⁰ (PM2 - Absent from controls (or at extremely low frequency if recessive) in Exome
8
9 Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium, PVS1 - Null
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11 variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single or multiexon
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13 deletion) in a gene where LOF is a known mechanism of disease). To confirm if the *RUNX1* CNV
14
15 deletion was present in all affected patients, Multiplex ligation-dependent probe amplification
16
17 (MLPA) analysis was performed which includes all exons of the
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19 *RUNX1/CEBPA/GATA2/TERC/TERT* genes (MRC Holland kit P437-B1), and can detect both
20
21 deletions and duplications. The index patient P1 and relatives P2 and P3 were all confirmed to
22
23 have the heterozygous *RUNX1* exon 3 – 7 deletion by MLPA analysis (Figure 1C). The deletion
24
25 removes 870bp of the mRNA sequence of *RUNX1* which is in frame, but completely removes the
26
27 DNA/Runt DNA binding domain and part of the activation domain (Figure 1D).
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32 Discussion

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34 This report describes a family with an inherited thrombocytopenia and history of excessive
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36 bleeding. FPDMM is confirmed in this family studied with the presence of a pathogenic germline
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38 *RUNX1* mutation¹¹⁻¹³. To date more than 70 FPDMM families have been reported with a 40%
39
40 lifetime risk of developing MDS and/or AML¹⁴. While the majority of familial cases reported in the
41
42 literature with *RUNX1* mutations are point mutations, different extended deletions have also been
43
44 described^{5, 11, 15, 16} (Figure 1D). Here using targeted NGS and subsequent CNV/MLPA analysis, we
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46 have detected a novel heterozygous deletion covering exons 3 – 7 of the *RUNX1* gene which is
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48 predicted to remove the entire DNA Runt/DNA binding domain. *RUNX1/AML1* encodes the DNA
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50 binding unit of the heterodimeric core binding factor (CBF) that is a critical regulator of definitive
51
52 hematopoiesis¹⁷. *RUNX1* is also recurrently mutated gene in sporadic myelodysplastic syndrome
53
54 and leukaemia. In sporadic AML, mutations in *RUNX1* are usually secondary events, whereas in
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56 FPDMM they are initiating events. None of the patients included in this study have developed AML
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58 as a result of a secondary somatic event occurring within *RUNX1* to date. It is thought that *RUNX1*
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3 variants causing haploinsufficiency are thought to be associated with a lower incidence of myeloid
4 malignancies when compared to those patients with dominant negative *RUNX1* defects. However
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6
7 the detection of a *RUNX1* variant in such patients with low platelet counts allows the possibility of
8
9 accurate counselling and clinical management for both them and extended family members at 50%
10
11 risk of inheriting the pathogenic variant, whom could go onto develop other cell malignancies.
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28
29 KR, BM and NVM extracted or generated clinical or experimental data; IA, KD, KF, JM, PP, KR
30
31 and NVM interpreted the results; KD, and NVM undertook governance of the study; BM and NVM
32
33 wrote the manuscript; all authors read and approved the final version of the manuscript.
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40

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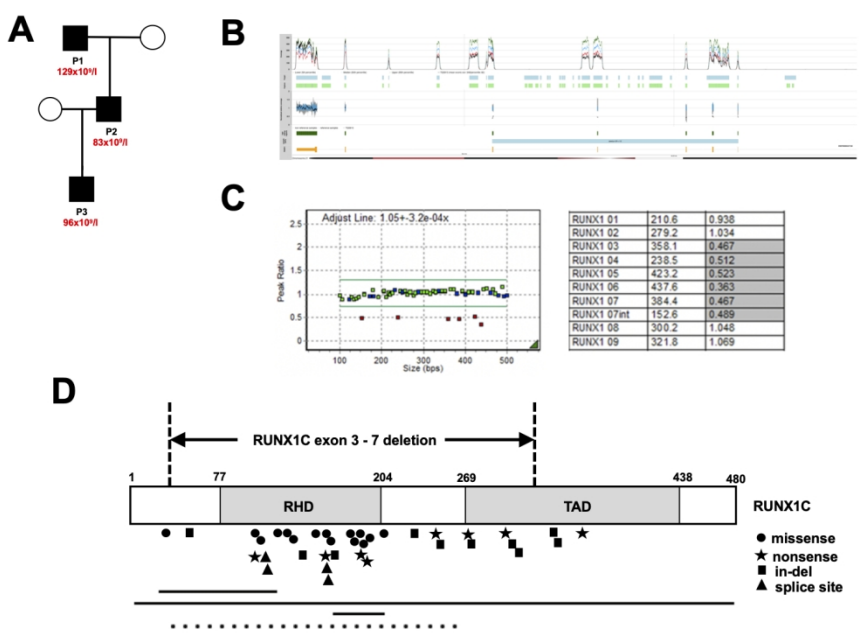
Table 1. Results of haematological analysis in patients P1-P3 at time of recruitment

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	$4.41 \times 10^{12}/L$	4.47	4.34	3.8 - 6.5
White cell count	$5.37 \times 10^9/L$	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	$129 \times 10^9/L$	$83 \times 10^9/L$	$96 \times 10^9/L$	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	$67.0 \times 10^9/L$ (1.52%)	$91.6 \times 10^9/L$ (2.05%)	$81.6 \times 10^9/L$ (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.

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