Thrombocytopenia WITH thromboxane A2 pathway AND dense granule secretion defect

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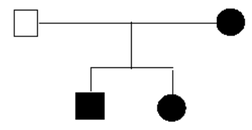
INTRODUCTION

- Mucocutanous bleeding symptoms, such as epistaxis and excessive bruising, are frequent in childhood.
- Despite an improvement in laboratory diagnostic tools for the investigation of platelet disorders, according to the Canadian Rare Inherited Bleeding Disorders Registry, the definitive diagnosis of the specific disorder in more than 50% of patients with mucocutanous bleeding and a platelet defect, remains undefined.
- Here, we present a family with an as yet undefined inherited platelet function defect affecting both platelet aggregation and secretion.

CASE PRESENTATION

- A 13 yr old boy (B) was referred to our clinic for further investigation because of easy bruising, recurrent epistaxis and a bleeding episode requiring surgical hemostasis after a minor cut.
- The family history also revealed easily bruising in the patient's younger sister (S; 7yr) and their mother (M; 45yr). The mother, in addition, has menorrhagia with periods lasting greater than 2 weeks, and experienced an episode of prolonged bleeding after a tooth extraction requiring hospitalization and repeated platelet transfusions.



- The Pediatric Bleeding Questionnaire bleeding score was abnormal (≥2) in all 3 individuals: B–8; S–4; M–18.

LABORATORY FINDINGS

Haematology:

	B (13 yr)	S (7 yr)	M (45 yr)
HGB	124 g/L	131 g/L	127 g/L
HCT	0.40	0.40	0.38
WBC	4.8 x10^9	5.8 x10^9	5.1 x10^9
PLT	**149** x10^9	**108** x10^9	**71** x10^9
MPV	7.6 fL	8.3 fL	7.2 fL
ABO/Rh	O pos.	O pos.	O pos.

The blood film of all subjects had an unremarkable platelet morphology and no neutrophil inclusion were observed.

Coagulation Parameters:

INR	1.1	1.1	1.0
aPTT	33 s	33 s	32 s
TT	15.9 s	15.6 s	*NA*
Fibrinogen	3.2 g/L	3.6 g/L	3.1 g/L
FVIII:C	1.67 IU/mL	0.9 IU/mL	1.04 IU/mL
VWF:Ag	1.41 IU/mL	0.64 IU/mL	0.97 IU/mL
VWF:RCo	1.79 IU/mL	0.74IU/mL	1.18 IU/mL

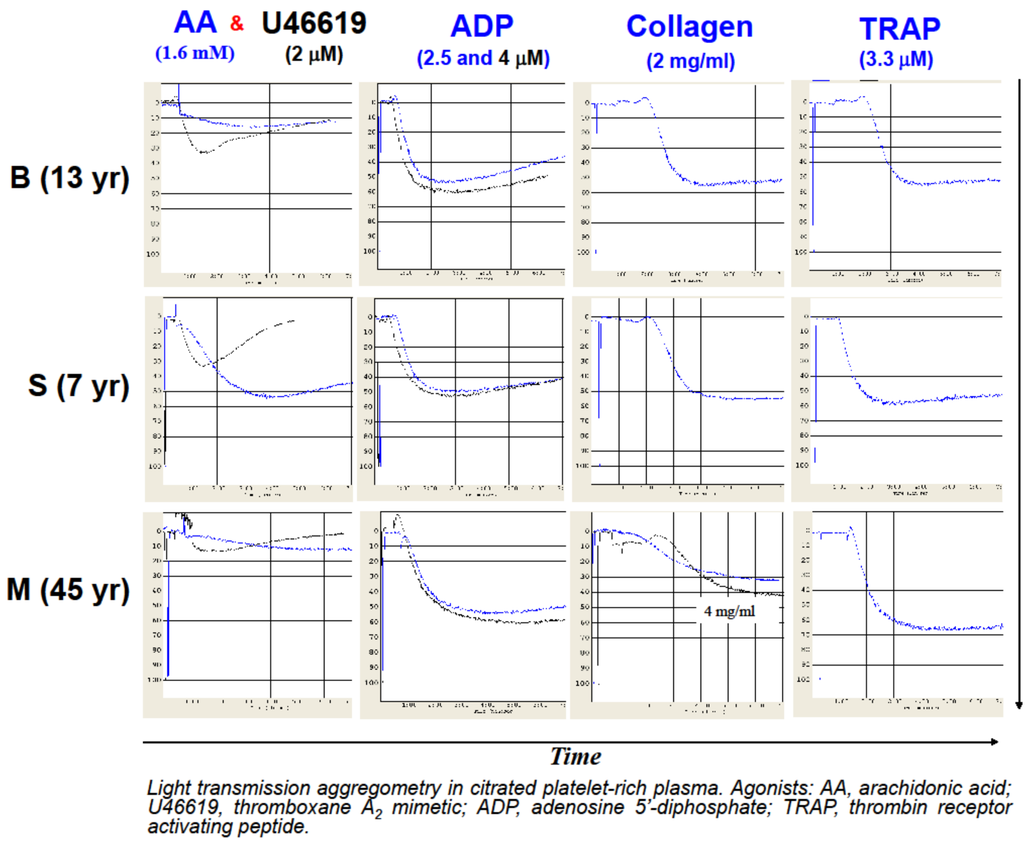
PFA-100 Closure Times:

Collagen/Epi	**>300** s	**>300** s	**>300** s
Collagen/ADP	117 s	119s	142 s

Upper limit of normal range for Collagen/Epinephrine cartridge: 168 s and for Collagen/ADP cartridge: 123 s.

→ Mild thrombocytopenia and greatly prolonged PFA-100 closure times with the collagen/epinephrine cartridge in all 3 individuals indicate the presence of an underlying inherited platelet function disorder.

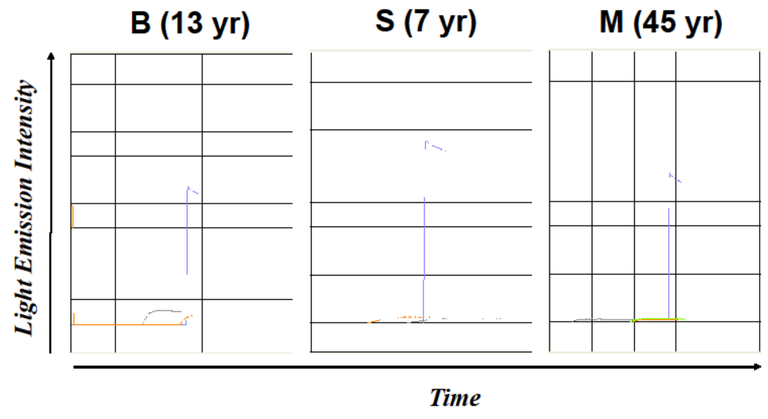
Platelet Aggregation:



Light transmission aggregometry in citrated platelet-rich plasma. Agonists: AA, arachidonic acid; U46619, thromboxane A_2 mimetic; ADP, adenosine 5'-diphosphate; TRAP, thrombin receptor activating peptide.

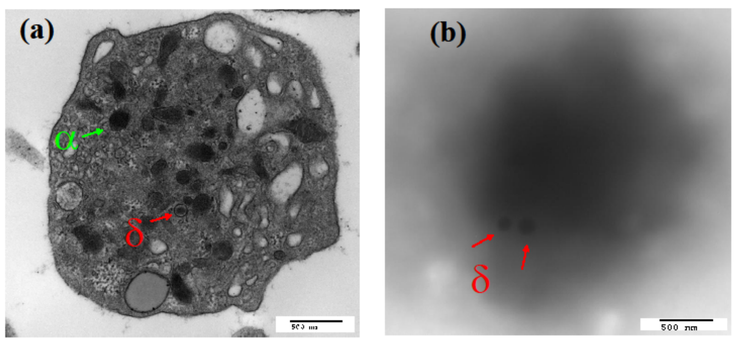
→ These findings indicate a platelet defect specifically involving the thromboxane pathway of aggregation.

Platelet Secretion:



Reduced thrombin (1U/mL)-induced ATP release from platelets measured using lumi-aggregometry (see black, orange or green tracings). Blue tracings represent ATP standard.

Transmission Electron Microscopy (TEM):



(a) Representative standard TEM image showing the ultra-structure of a platelet. α, alpha granule; δ, dense granule.
(b) Representative whole mount TEM image visualizing dense (δ)-granules.

The mean number of δ–granules per platelet in all 3 affected family members was only slightly reduced:
B – 1.7; S – 2.4; M – 1.2
(→ lower limit of normal – 3).

→ Impaired ATP release in response to thrombin, despite the presence of dense (d) granules, indicates a primary platelet secretion defect independent of the thromboxane pathway aggregation defect.

Platelet transcriptome analysis:

The RNA was extracted and processed as per *Rowley et al. Blood. 2011; Oct 6; 118(14):e101-11.* The following genes are currently being analyzed amongst other potential candidate genes:

- *DDT* = D-dopachrome tautomerase
- *DPH5* = diphthamide biosynthesis 5
- *DUSP3* = dual specificity phosphatase 3
- *EPHB2* = EPH receptor B2
- *ZNF804A* = zinc finger protein 804A
- *ERCC5* = excision repair cross complementation group 5

SUMMARY & CONCLUSIONS

- We have identified a family, a mother and her two children, with a mucocutanous bleeding disorder associated with:
  - mild thrombocytopenia;
  - impaired platelet aggregation affecting the thromboxane pathway; and
  - a platelet secretion defect.
- This platelet defect, that is inherited in an autosomal dominant fashion, likely involves defects in a gene/genes involved in platelet signal transduction pathways, and the maturation process of platelets during thrombopoiesis or the platelet life span.

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Platelet Disorders
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