# ERN-EuroBloodNet Topic on Focus on Inherited Platelet Function Disorders (IPFD)

webinar

HEALTH PROFESSIONALS

## Diagnostic and management of dense granule deficiency including Hermansky-Pudlak syndrome

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CRPP Centre de Référence Pathologies Plaquettaires
MHEMO La Filière des maladies rares de l'hémostase
ebn ERN | EuroBloodNet

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| Consultancy | Name of Institutes/Companies |
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| Conference fees | No conflicts of interest |
| Advisory Comitee member | No conflicts of interest |
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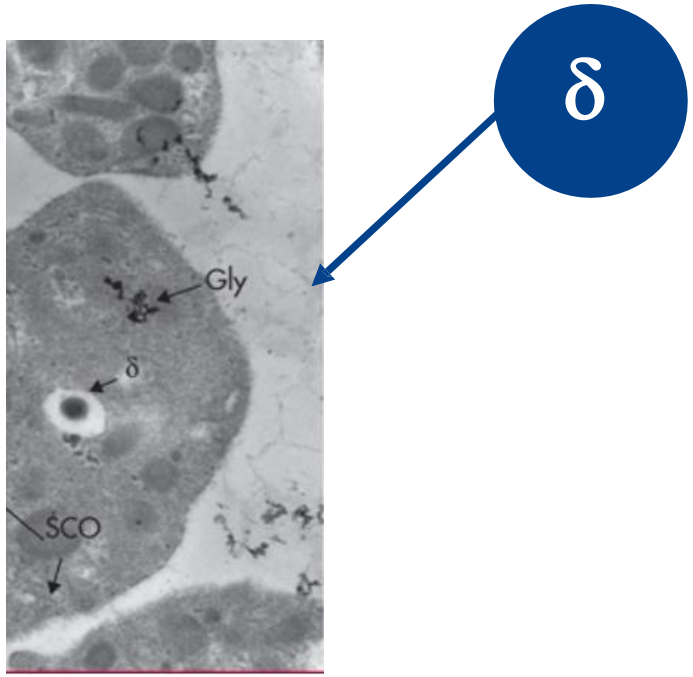
| | |

# Dense Granules




## Dense granules (3-8/platelet)

**Ca2+** at very high concentration (about 2 M)

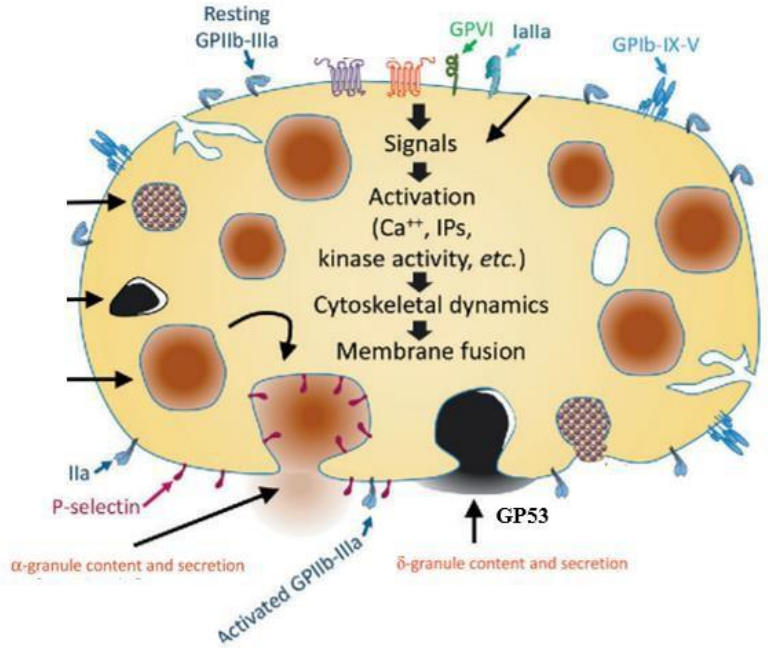
**ATP and ADP** at high concentrations of 0.6 and 0.4 M

**90% of the circulating serotonin** in the body (400 to 600 ng/$10^9$ platelets)

**Histamine…**

**Receptors (CD63, Lamp2, IIbIIIa, GPIb)**

**Coagulation,**
**Amplification of platelet activation**




*AD Mumford, Thromb Haemost, 2015*

# Dense Granule Deficiency (DGD)

- ❑ Heterogeneous subset of inherited platelet function disorders
- ❑ Quantitative defect: absence or a reduction in the number of dense granules
- ❑ And/or Qualitative defect: reduced content of some components

Reduced platelet activation
Bleeding disorder often moderate

*Orsini S, Haematologica 2017*

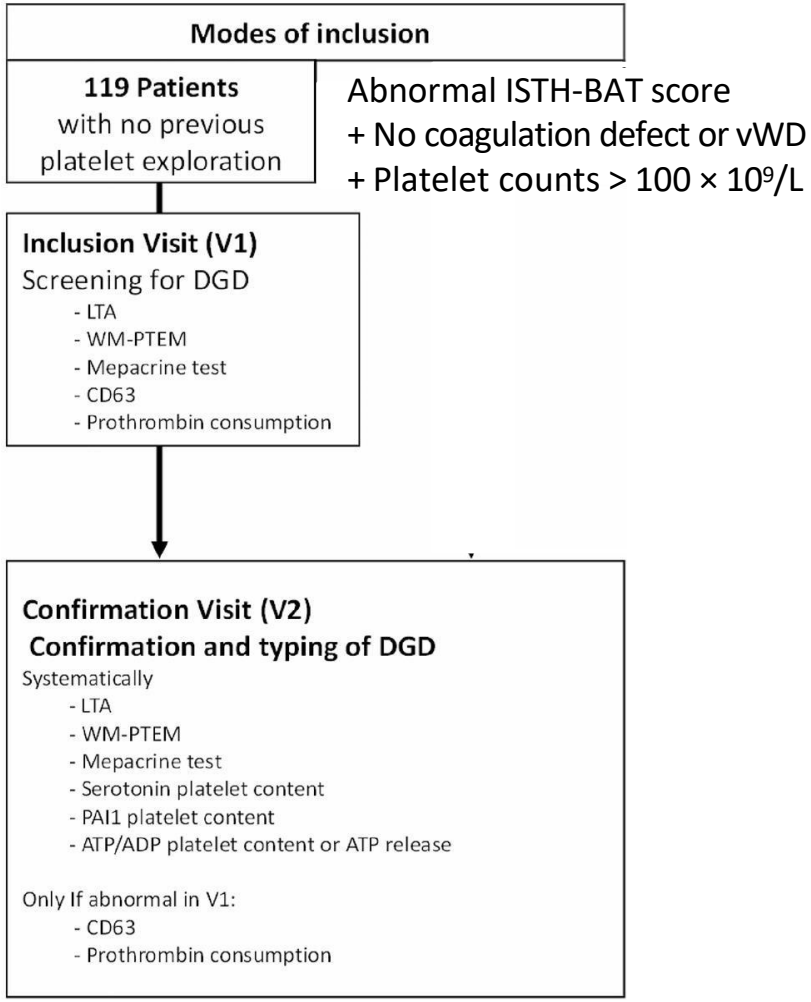
# Prevalence of DGD

- ❑ High, probably underestimated
- ❑ ~ Similar to VWD
- ❑ Depends on
  - population study

    The prevalence of Hermansky-Pudlak syndrome (HPS):
    - in Puerto-Rican population: 1/1,800
    - in general population: 1/350,000 to 1/1,000,000

  - sensitivity/specificity of platelet function tests:

    French experience : DGD prevalence ranged from 7.5% (≥2 abnormalities) to 37.4% (≥1 abnormality)

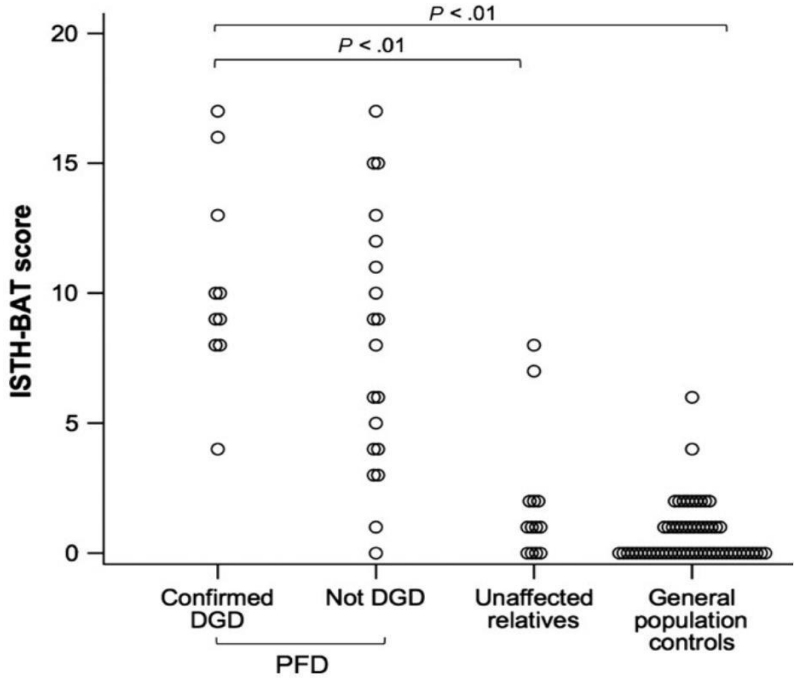




D Borgel, RPTH, 2026
A Bourguignon, Crit Rev Clin Lab Sci., 2022
Quiroga, *Br J Haematol*, 2009
Hayward, *J Thromb Haemost*, 2009
Seravalli, *J Pediatr Adolesc Gynecol*, 2013
Vo, *Haemophilia*, 2013
Hayward, *Semin Thromb Hemost*, 2012
Israels, *Br J Haematol*, 1990

# Relationship between DGD and bleeding

## ISTH-BAT scores distribution



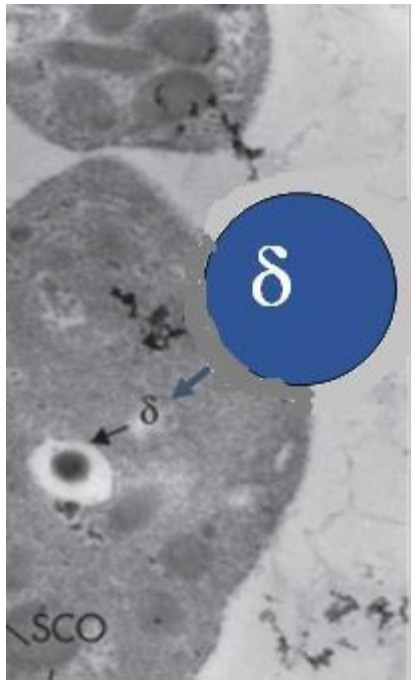

- Higher ISTH-BAT bleeding scores for individuals with confirmed DGD than unaffected relatives and general population controls.
- ISTH-BAT scores were not significantly different for individuals with platelet function disorder whether or not they carry DGD.

| | IPFD | N | Median ISTH-BAT (IQR) | ISTH-BAT Median (IQR) F<br>Median (IQR) M | ISTH-BAT Min-max F<br>Min-max M |
|---|---|---|---|---|---|
| | Glanzmann thrombasthenia | 79 | 11 (8-16) | 12 (8-16)<br>10 (7-13.5) | 1-26<br>4-20 |
| 10.7% | **d-storage pool deficiency** | **21** | **6 (3.75-10.5)** | **6 (5-13.5)**<br>**4 (2.75-9.25)** | **1-17**<br>**1-16** |
| | Biallelic Bernard Soulier syndrome | 20 | 8.5 (7.5-12.5) | 8.5 (8-11.5)<br>8 (1-12.75) | 4-27<br>0-16 |
| | Primary secretion defect | 20 | 7.5 (3.5-12.5) | 12 (7-14)<br>6 (0.5-6) | 1-21<br>0-11 |
| | Familial platelet disorder associated with myeloid malignancy | 8 | 4.5 (1-5.5) | 4.5 (3.5-6.2)<br>2.5 (0-5.2) | 2-10<br>0-6 |
| | Gray platelet syndrome | 7 | 12 (10-14.25) | 15 (13.5-15.5)<br>10 (9.5-10.5) | 12-16<br>8-12 |
| 3.5% | **Hermansky-Pudlak syndrome** | **7** | **5 (2-13.25)** | **6.5 (2.75-13.2)**<br>**1 -** | **2-18**<br>**1-1** |
| | Combined alpha-delta granule deficiency | 5 | 8 (5.75-9) | 8.5 (6.5-9)<br>7 - | 2-9<br>7-7 |

- **Moderate cutaneomucous bleeding disorder vs other PFD** : epistaxis, bruising, bleeding from minor wounds and oral cavity, hematuria, tooth extraction, surgery, gynecological bleeding, muscle hematomas, central nervous system bleeding, others.

J.G *Brunet*, Int J Lab Hem. 2018
P Gresele, JTH, 2020

# Conditions associated with DGD

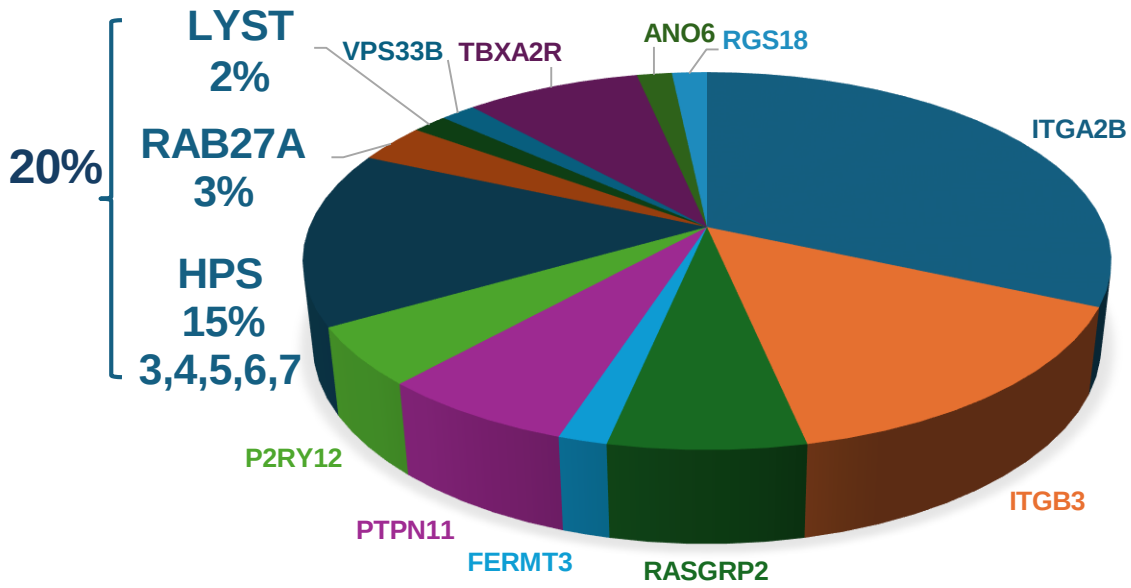



**Syndromic forms**
**Associated with deficiency of other granules (skin melanosomes, leukocyte granules)**

oculocutaneous depigmentation
immune deficiencies


Hermansky–Pudlak syndrome (*HPS1-11*)
Chediak–Higashi disease (*LYST*)
Griscelli syndrome (*RAB27A*)

**Marseille 2014-2024: 58/298 (20%) *cases platelet function disorder & pathogenic variants***




*Dupuis A, J Clin Med. 2020*
*Gunay-Aygun M, Semin Thromb Hemost. 2004*
*Huizing M, Thromb Haemost. 2001*
*Rendu F, Am J Pathol. 1983*

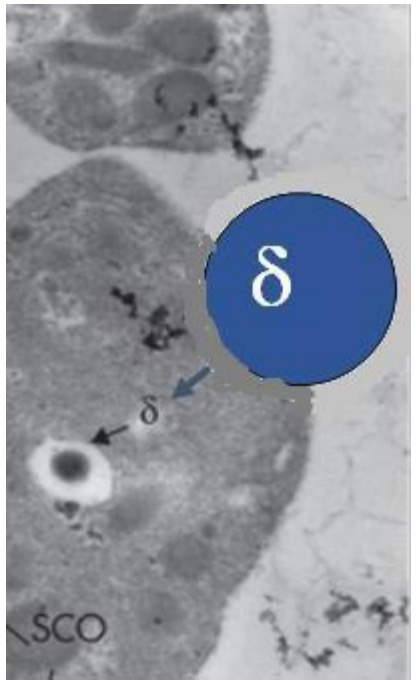
# Diagnosis of HPS can be challenging

Variable hypopigmentation ranging from complete absence of pigment to nearly normal melanin production → clinical presentation can be unclear (HPS3, HPS5, HPS6)

All HPS patients suffer from ophthalmological disorders (nystagmus, foveal hypoplasia, optic nerve decussation anomalies, retinal hypopigmentation, and decreased visual acuity)

→ Important to seek the opinion of an ophtalmologist in case of severe DGD

# Conditions associated with DGD

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**Associated with inherited thrombocytopenia**

*RUNX1*
*GATA1*
*FLI-1*

*SLFN14*
*FLNA*
*GFI1B*

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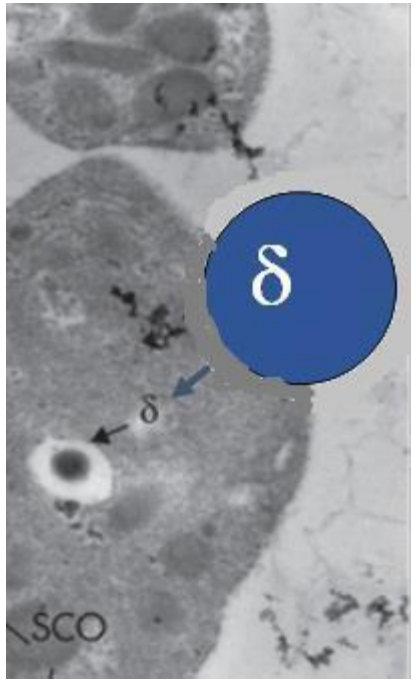


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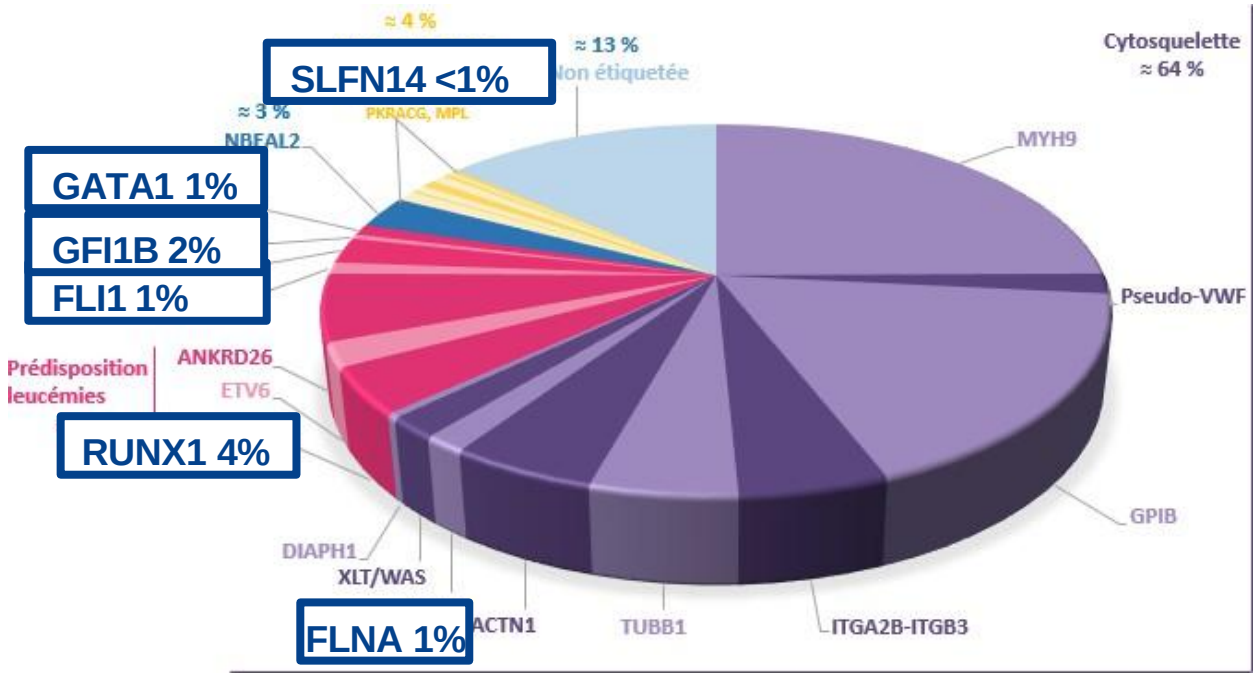


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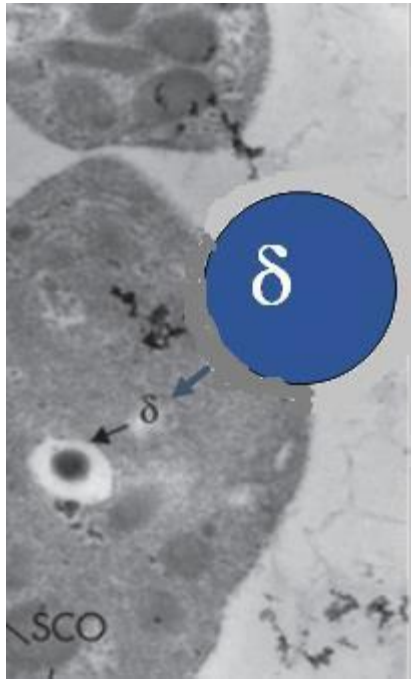


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**Associated with acquired conditions**

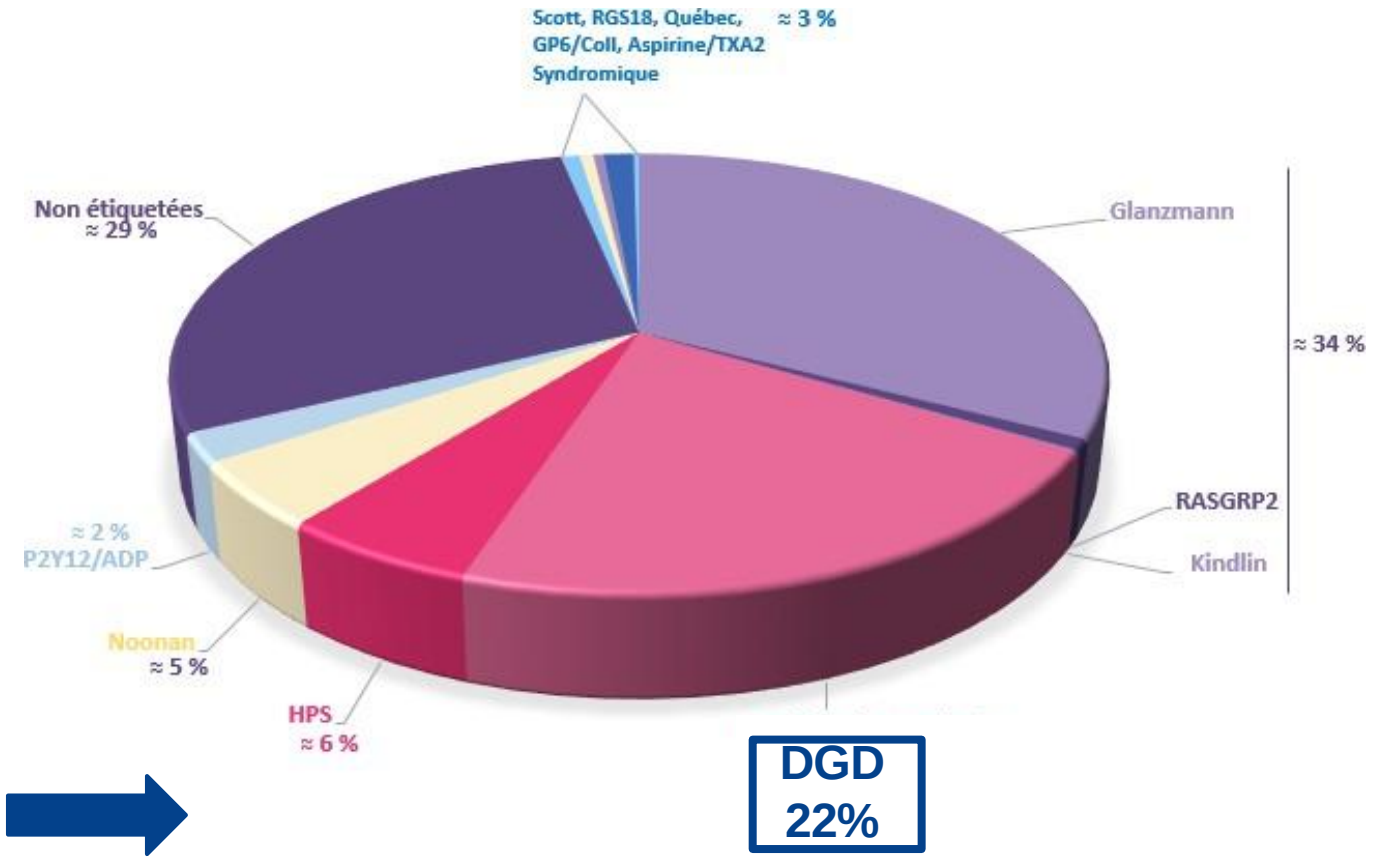
Serotonin reuptake inhibitors

**Undetermined etiology**

*Dupuis A, J Clin Med. 2020*
*Gunay-Aygun M, Semin Thromb Hemost. 2004*
*Huizing M, Thromb Haemost. 2001*
*Rendu F, Am J Pathol. 1983*

# Undetermined etiology of DGD

**898 patients carrying platelet function disorder care card 2025**




❑ DGD of undetermined etiology represent the second most common platelet dysfunction after Glanzmann Thrombasthenia.

# δ-Platelet dense granule assessment: when ?

- A suggestive syndromic form
- Bleeding despite mild thrombocytopenia
- Unexplained bleeding disorders
  - Mucocutaneous bleeding
  - Unexplained surgical bleeding
  - Iron deficiency and anemia

Performed tests as a second diagnostic step or at first line test after ruling out vWD

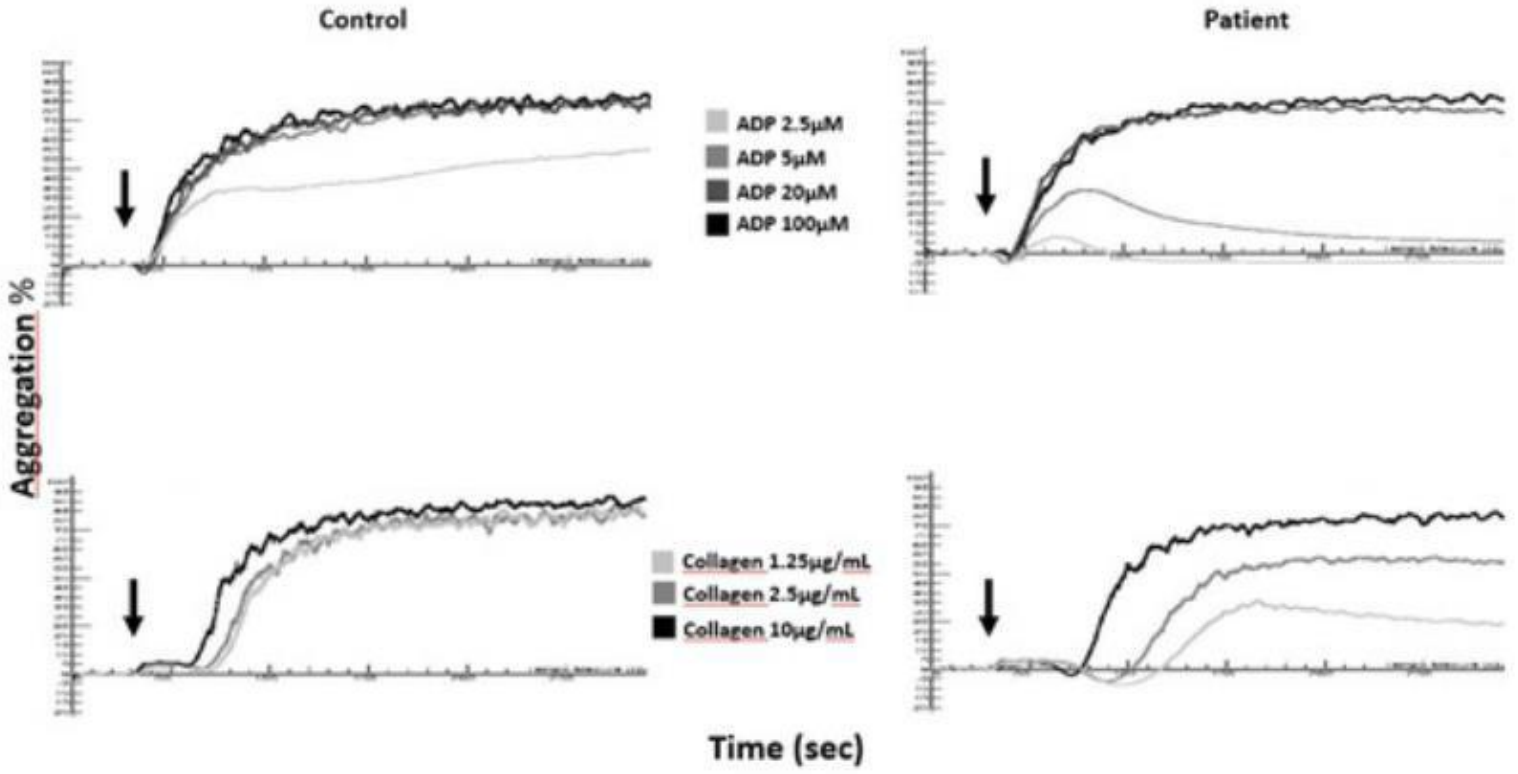
*P Gresele, JTH, 2014, 2015*
*D Mezzano, JTH, 2022*

# How to diagnose DGD

## Indirect tool

❑ Platelet aggregation

- reduced aggregation and secretion in response to low-dose activators
- lack of a second wave of aggregation in response to ADP and epinephrine
- decreased response to collagen



# How to diagnose DGD

## Indirect tool

❑ Platelet aggregation

- reduced aggregation and secretion to low concentrations of low-dose agonists
- lack of a second wave of aggregation with ADP and epinephrine
- decreased response to collagen

Light Transmission Aggregometry is not sensitive enough

*Nieuwenhuis, H.K. Blood 1987*
*Quiroga Br J Haematol 2009*
*Brunet JG Int J Lab Hematol 2018*
*Dupuis A, J Clin Med. 2020*
*Gunning WT, J hematol 2021*

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## More specific tools

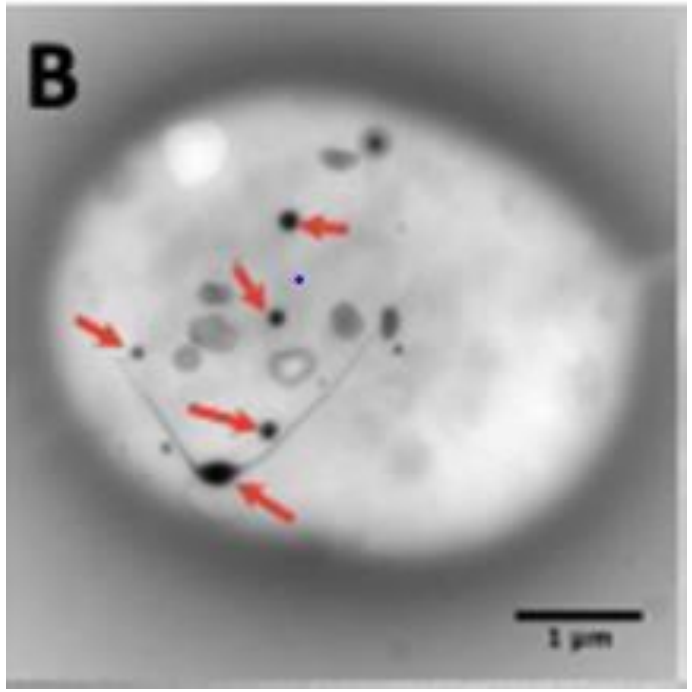
❑ Dense granule quantification

❑ Dense granule content assessment (before and after activation)

# Dense granule quantification

## Whole Platelet Mount

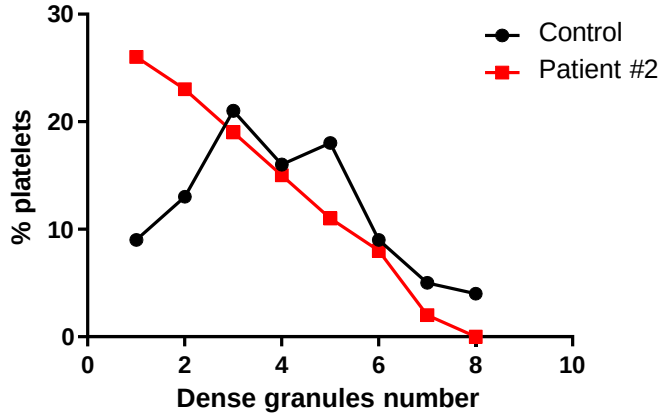
Transmission electron microscopy of unstained and unfixed platelets




*BS Bull, Blood, 1966*

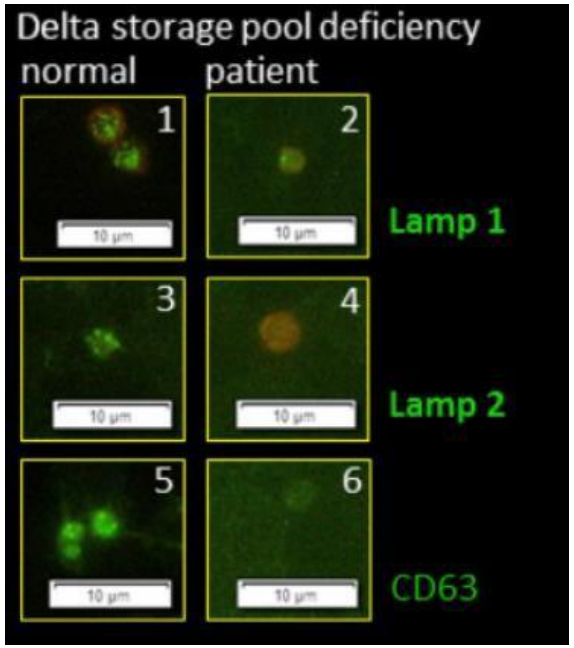
## Fluorescence microscopy

### Mepacrine uptake




Personal data

### Immunofluorescence




A Greinacher, JTH, 2017

# Dense granule evaluation by flow cytometry

## Mepacrine uptake and release

- ❑ Mepacrine uptake showed high NPP = 97%
- ❑ Can be used to exclude DGD in patients with a bleeding tendency

## CD63 before and after activation

- ❑ Very low level in the basal state
- ❑ Is also present in lysosomes
- ❑ Does not allow to distinguish secretion defect from a dense granule deficiency

**An ongoing project conducted by Sofia Ramström and Dianne Van Der Wal (SSC on platelet physiology)** aims to better define the diagnostic value of flow cytometry, alongside established methods

*Cai H, Semin. Thromb. Hemost. 2016*
*Van Asten I, J. Thromb. Haemost. 2020*

# Dense granule content

- **Quantification of ATP and ADP** (luminescence, HPLC)
  - Basal (ATP/ADP ratio > 4 ) and released after activation (collagen, AA, ADP, TRAP)

*AD Mumford, Thromb. Haemost, 2015*

- **Quantification of serotonin** (ELISA, HPLC, LC-MS)
  - Basal (in platelet lysates) and released after activation
  - Uptake and Release of radio-Labeled Serotonin (abandoned historical method)

*P Guicheney, Exp. Clin. Pharmacol, 1988*
*E Flachaire, Clin. Chem, 1990*
*GM Anderson, Anal. Biochem 1992*

**ISTH SSC recommandation**: There was consensus that HPLC-based measurement of **ADP/ATP, and serotonin should be considered the gold standard**, as it provides high sensitivity and specificity, and allows simultaneous evaluation of total content and secretion

D *Mezzano , JTH, 2022*

# DGD management

## High bleeding rates in DGD during surgeries and deliveries BEFORE DIAGNOSIS:

- Before the diagnosis of DGD, 35 of 68 interventions and deliveries (51.5%) were associated with bleeding events.

### Interventions

| Treatment group | Total *n* | No bleeding *n* (%) | Minor bleeding *n* (%) | Major bleeding *n* (%) | Total bleeds *n* (%) |
|---|---|---|---|---|---|
| **Before diagnosis** | | | | | |
| Without preventive treatment | 57 | 30 (52.6) | 25 (43.9) | 2 (3.5) | 27 (47.4) |
| With preventive treatment[a] | 4 | 1 (25) | 3 (75) | 0 | 3 (75) |
| **After diagnosis** | | | | | |
| Without preventive treatment | 20 | 15 (75) | 4 (20) | 1 (5) | 5 (25) |
| With preventive treatment[a] | 64 | 56 (87.5) | 7 (10.9) | 1 (1.6) | 8 (12.5) |

[a]Preventive treatment includes tranexamic acid, desmopressin, platelet transfusion, or a combination of these.

### Deliveries

| Treatment | Total *n* | No bleeding *n* (%) | PPH *n* (%) |
|---|---|---|---|
| **Before diagnosis** | | | |
| Without preventive treatment | 5 | 0 | 5 (100) |
| With preventive treatment[a] | 2 | 2 (100) | 0 |
| **After diagnosis** | | | |
| Without preventive treatment | 1 | 0 | 1 (100) |
| With preventive treatment[a] | 8 | 5 (62.5) | 3 (37.5) |

PPH, postpartum hemorrhage.
[a]Preventive treatment includes tranexamic acid, desmopressin, platelet transfusion, or a combination of these.



*E V Heerwaarde, RPTH, 2025*

# DGD management

## High bleeding rates in DGD during surgeries and deliveries BEFORE DIAGNOSIS:

- ❑ Before the diagnosis of DGD, 35 of 68 interventions and deliveries (51.5%) were associated with bleeding events.
- ❑ After the diagnosis of DGD, 17 of 93 interventions and deliveries (18.3%) were associated with bleeding events.

### Interventions

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PPH, postpartum hemorrhage.
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*E V Heerwaarde, RPTH, 2025*

# DGD management

## High bleeding rates in DGD during surgeries and deliveries BEFORE DIAGNOSIS AND WITHOUT PREVENTIVE TREATMENT:

- ❑ Bleeding rates are significantly lower among patients who received preventive treatment.

### Minor Interventions

| Minor interventions | Total *n* = 63 | Minor bleeding event | Major bleeding event | Total bleeds |
|---|---|---|---|---|
| Without preventive treatment | 38 (60.3) | 10 (26.3) | 0 | 10 (26.3) |
| With preventive treatment | | | | |
| TXA only | 9 | 0 | 0 | |
| DDAVP only | 4 | 1 | 0 | |
| TXA + DDAVP | 4 | 0 | 0 | |
| PT | 7 | 1 | 0 | |
| TXA + DDAVP + PT | 1 | 0 | 0 | |
| Total | 25 (39.7) | 2 (8.0) | | 2 (8.0) |

### Major Interventions

| Major interventions | Total *n* = 82 | Minor bleeding event | Major bleeding event | Total bleeds |
|---|---|---|---|---|
| Without preventive treatment | 39 (47.6) | 19 (48.7) | 3 (7.7) | 22 (56.4) |
| With preventive treatment | | | | |
| DDAVP only | 7 | 4 | 0 | 4 |
| TXA + DDAVP | 9 | 2 | 0 | 2 |
| PT | 23 | 1 | 1 | 2 |
| TXA + PT | 2 | 0 | 0 | 0 |
| DDAVP + PT | 1 | 1 | 0 | 1 |
| TXA + DDAVP + PT | 1 | 0 | 0 | 0 |
| Total | 43 (52.4) | 8 (18.6) | 1 (2.3) | 9 (20.9) |

### Deliveries

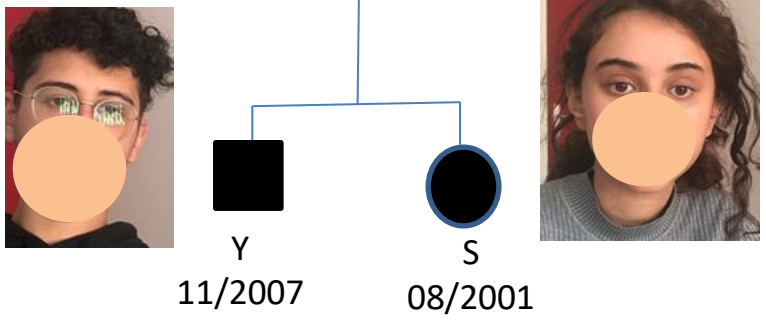
| Vaginal deliveries | Total *n* = 10 | PPH |
|---|---|---|
| Without preventive treatment | 6 | 6 (100) |
| TXA + DDAVP | 4 | 3 (75) |
| **Cesarean sections** | **Total *n* = 6** | **PPH** |
| PT | 6 | 0 |

TXA: tranexamic acid;
DDAVP: desmopressin;
PT: platelet transfusion;
PPH: postpartum hemorrhage

**Early diagnosis and appropriate preventive management reduce bleeding complications in patients with DGD.**

*E V Heerwaarde, RPTH, 2025*

# Diagnosis and management of severe DGD : Real life experience




| | Y | S |
|---|---|---|
| **ISTH-BAT score** | 7 | 2 |
| Gingival bleeding | | |
| Bruising | | |
| Epistaxis | | |
| Post surgical bleeding* | | |
| Abnormal uterine bleeding | | |

* tonsillectomy/ adenoidectomy, circumcision

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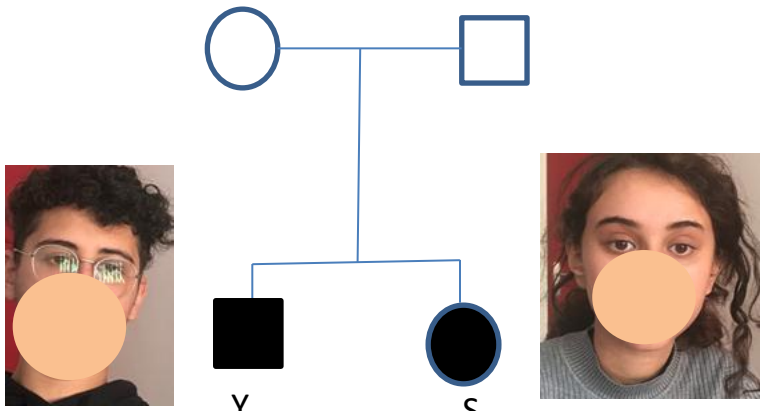


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- Severe DGD :

- Normal light transmission aggregometry.
- Decreased CD63 expression after 10 µM ADP, and 50µM TRAP only in S (normal CD63 expression after TRAP for Y).
- Decreased mepacrine uptake by flow cyometry and fluorescence microscopy.
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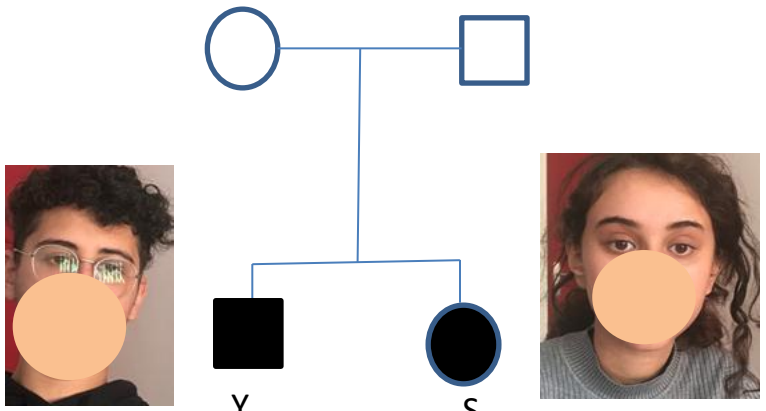


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| **Non-hematological features** | | |
| Nystagmus | | |
| Ocular albinism | | |

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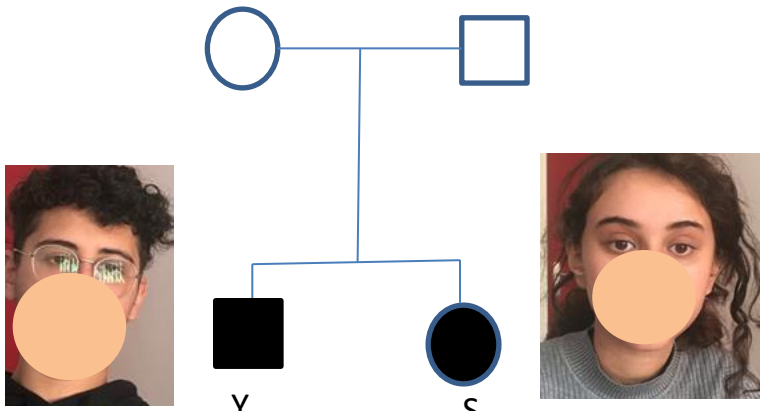


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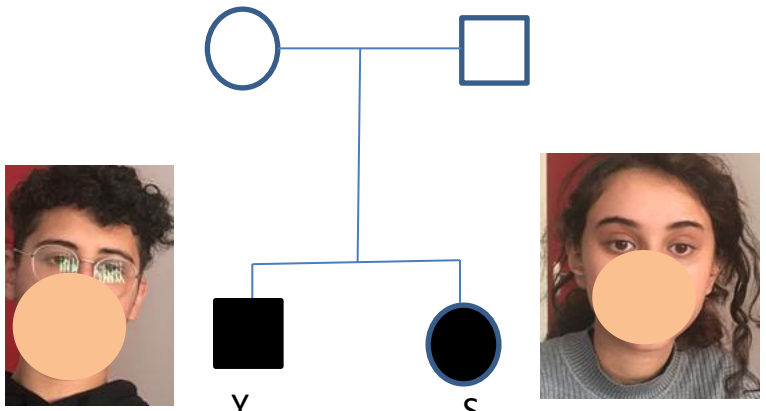
Homozygous pathogenic variant *HPS6*

# Diagnosis and management of severe DGD : Real life experience




| | Y | S |
|---|---|---|
| **ISTH-BAT score** | 7 | 2 |
| Gingival bleeding | | |
| Bruising | | |
| Epistaxis | | |
| Post surgical bleeding* | | |
| Abnormal uterine bleeding | | |
| **Non-hematological features** | | |
| Nystagmus | | |
| Ocular albinism | | |

* tonsillectomy/ adenoidectomy, circumcision

❑ Severe DGD :

- Normal light transmission aggregometry.
- Decreased CD63 expression after 50µM TRAP and 10 µM ADP (normal CD63 expression after TRAP for Y).
- Decreased mepacrine uptake by flow cyometry and fluorescence microscopy
- Decreased platelet serotonin content

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❑ Management of a third molar extraction for Y :

- Care at the university hospital
- Local hemostasis optimized
- Tranexamic acid + Desmopressin immediately before surgery and after 24 hours
- No bleeding complication was observed

# Take Home Messages
# Dense Granule Deficiency

- ❑ Frequent condition
- ❑ Syndromic forms: consultation with ophtalmologist is mandatory
- ❑ Associated with thrombocytopenia / acquired (e.g. reuptake serotonin inhibitor)
- ❑ Moderate spontaneous bleeding
- ❑ Higher perioperative & peripartum bleeding rates
- ❑ Earlier diagnosis and preventive treatment could decrease this risk

# Take Home Messages

- ❑ Numerous tools available for DGD diagnosis, but in practice their use remains limited due to a lack of standardization, validation, and also cost constraints

According to an ISTH survey, half of laboratories do not evaluate platelet granules

*[Gresele J. Thromb. Haemost 2014].*

- ❑ No validated recommendations to guide the diagnostic strategy or prioritize tests

- ❑ In the meantime, a pragmatic approach could be proposed:

  - ✓ Each laboratory select methods adapted to their local setting,
  - ✓ Combining tests is likely to improve diagnostic accuracy,
  - ✓ Confirmed results on several occasions

# THANK YOU

CRPP Centre de Référence Pathologies Plaquettaires

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