Phenotypic and Genotypic Characterization of MYH9 related macrothrombocytopenia in four Portuguese Families

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INTRODUCTION

Congenital thrombocytopenias are a group of rare and heterogeneous diseases. Within these entities, there are familial thrombocytopenias characterized by an autossomal dominant inheritance pattern and macrocytic platelets. The main mechanism of thrombocytopenia consists in quantitative and/or qualitative defects of proplatelet formation, while megakaryocyte differentiation and maturation are essentially preserved. In most cases these thrombocytopenias derive from mutations in genes for components of the acto-myosin cytoskeleton[1]. Macrothombocytopenia diseases related to mutations in the myosin heavy chain protein (MYH9), including platelet disorders such as the MayHegglin anomaly and the Epstein, Fechtner, and Sebastian syndromes, may be more common than previously thought. These syndromes share different clinical manifestations such as bleeding, nephropathy, sensorineural hearing loss and cataracts. The clinical characteristics may vary among individuals within the same family or sharing the same mutation.

AIM

The aim of this study was to describe the phenotypic and genotypic characteristics of four families with MYH9-related macrothrombocytopenia (MYH9-RM).

METHODS

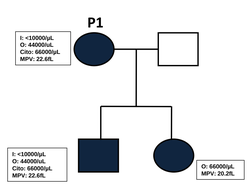
Nine individuals with macrothrombocytopenia from 4 unrelated families were studied. Clinical presentation with focus on haemorrhagic manifestations and inherited pattern, were evaluated. Laboratory evaluation included platelet count (impedance and optical analysis, flow-cytometric analysis and manual counting); platelet indices (mean platelet volume – MPV and immature platelet fraction – IPF); Platelet and leukocyte (Dohle-like bodies) morphology were evaluated on blood films stained with Giemsa and observed under light microscopy. Transmission electron microscopy studies were also performed. Platelet function was tested by closure time (CT) with the Platelet Function Analyzer (PFA) and light transmission aggregometry (LTA). The levels of platelet glycoprotein (CD41, CD61 and CD42b expression) were evaluated by flow cytometry (FCM). Fourteen coding regions of the *MYH9* gene known to be mutational hotspots were studied by direct sequencing.

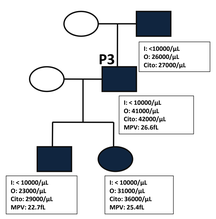
RESULTS

Clinical manifestations

Two families (P1 and P3) with autosomal dominant inheritance pattern. Two families (P2 and P4) with sporadic macrothrombocytopenia.

Patient	Sex	Age at diagnosis	Bleedind severity	Related situations	Extra-hematologic manifestation
P1	Female	25	Mild	Previous ITP diagnosis at 16 years old	None
P2	Female	3	Mild	Corticoid therapy after head trauma	None
P3	Male	38	Mild	Cervical surgery under desmopressin without bleeding	None
P4	Female	29	Moderate	ITP at 12 years-old without response to corticoid and splenectomy	Nephropathy, cataracts and deafness detected at 23 years-old



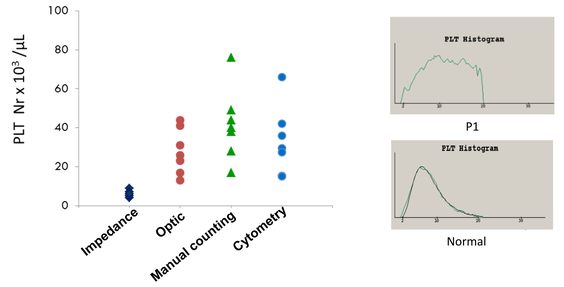


Laboratory evaluation

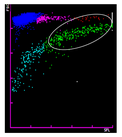
PLATELET COUNT

- Impedance analysis: <10x10^3/µL, one patient 82x10^3/µL
- Optical analysis: 5 - 139x10^3/µL
- Flow-cytometric analysis: 15-66x10^3/µL
- Manual counting: 13 - 76x10^3/µL

MEAN PLATELET VOLUME: 18.8 - 27.5 fL (N: 7 – 11fL)

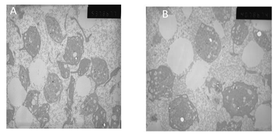


IMMATURE PLATELET FRACTION



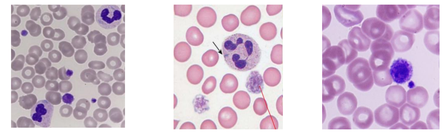
IPF: 61.3 ± 11.1 % (N: 3.9 ± 1.2 %)

TRANSMISSION ELECTRON MICROSCOPY



A – Normal (x5300) ; B – Patient (x4000)

MORPHOLOGY



Platelet macrocytosis and giant platelets; presence of Dohle-like bodies in neutrophils, in 4 patients.

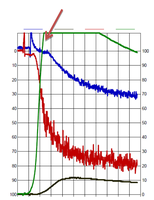
PLATELET FUNCTION TESTS

- **PFA:** Closure times were normal or slightly increased.

Col/ADP 85/137s (N:57-127 s)

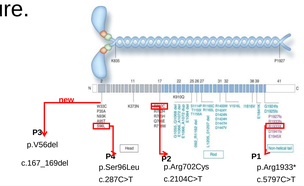
Col/Epi 116/150s (N:88-15 s)

- **Flow cytometry**: Normal expression of glycoproteins
- **Light transmission aggregometry:** Absent shape change in the aggregation curve with collagen.



MYH9 MUTATION ANALYSIS

Three families presented different known mutations and one family presented a variant in exon 2 (c.167_169del) not previously reported in the literature.



CONCLUSION

The MYH9-RM are often misdiagnosed as ITP, especially if isolated thrombocytopenia is present. In the presence of severe thrombocytopenia, variables platelets counts according to the hematologic analyser, macrocytosis and giant platelets, and normal or slightly altered platelet function, MYH9-RM should be suspected. New platelet parameters such as IFP seams to be promising. The identification of mutations confirms the clinical diagnosis. Recognition of this entity avoids misdiagnosis and inappropriate therapeutics (immunosuppressive treatments or even splenectomy) and, in syndromic cases, it enables earlier awareness of associated manifestations (nephropathy, deafness or cataracts).

BIBLIOGRAPHY

1 – Pecci, A; Pathogenesis and management of inherited thrombocytopenias: rationale for the use of thrombopoietin-receptor agonists, *Int. Journal Hematology,* 2013, 98:34-47