The Official Journal of the Italian Society for the Study of Hemostasis and Thrombosis
SISET
Società Italiana per lo Studio dell'Emostasi e della Trombosi
Corso Nazionale SISET
Bergamo | 23-25 ottobre 2025

29TH NATIONAL CONFERENCE OF THE ITALIAN SOCIETY FOR THE STUDY OF HEMOSTASIS AND THROMBOSIS, 2025

ABSTRACT N. CO40

ALTERAZIONI DELLE PIASTRINE E CONDIZIONI GENETICHE

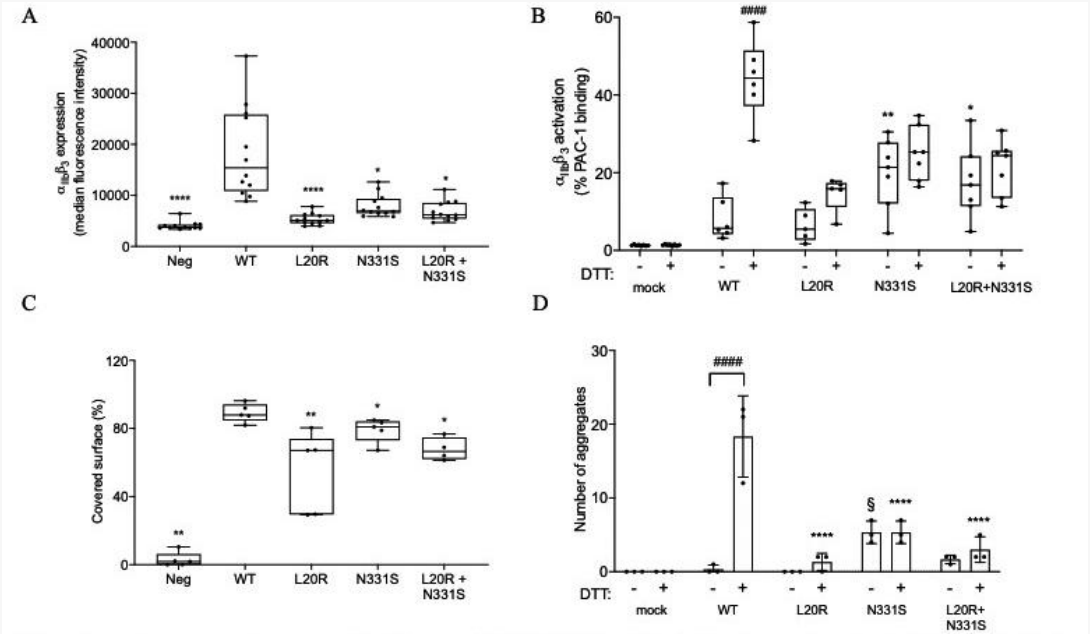
THE CO-INHERITANCE OF TWO ITGB3 VARIANTS EXERTING ADDITIVE DETRIMENTAL EFFECTS ON PLATELETS LEADS TO VARIANT GLANZMANN THROMBASTHENIA.

N. Arndt[1], E. Falcinelli[1], A. Zamora-Canovas[2], A. Sánchez Fuentes[2], A. Marín-Quilez[2], I. Tano[1], M.D.M. Nieto-Hernández[3], J.M. Bastida[4], J. Rivera[2], P. Gresele[1], L. Bury[1].

1Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine, University of Perugia, Perugia; [2]Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Pascual Parrilla, ISCIII--CIBERER, Murcia, Spain; [3]Servicio de Hematología, Hospital Universitario de Jaen, Jaén, Spain; [4]Departamento de Hematología, Complejo Asistencial Universitario de Salamanca "CAUSA", Instituto de Investigación Biomédica de Salamanca "IBSAL", Centro de Investigación del Cáncer, IBMCC-CSIC, Universidad de Salamanca "USAL", Salamanca, Spain.

Background and Aims
Glanzmann thrombasthenia (GT) is an autosomal recessive platelet disorder with reduced αIIbβ3 expression or function and normal platelet count, caused by a reduced expression or dysfunction of integrins αIIb or β3 encoded by the genes *ITGA2B* and *ITGB3* respectively. Dominant gain-of-function (GOF) variants in *ITGA2B* or *ITGB3* cause macrothrombocytopenia with platelet dysfunction. The co-inheritance of two heterozygous αIIbβ3 gene variants with different impact on integrin function is a rare event whose final effect on platelet function is hardly predictable.
We previously described a patient with variant GT characterized by macrothrombocytopenia and a partial platelet aggregation defect with mild clinical bleeding due to the co-inheritance of a GOF p.Asn331Ser and a LOF *ITGB3* variants, with the GOF exerting a dominant effect over the wild type and LOF β3. Here we report an unusual new case in which the co-inheritance of a GOF and a LOF variant in *ITGB3* associated with a severe GT clinical phenotype
Methods
Patient platelet function was assessed and DNA sequenced by HTS-gene panel. Expression vectors were generated, and αII-bearing CHO cells were transfected with p.Asn3331Ser, p.Leu20Arg, or both β3 variants.
CHO cells were studied by confocal microscopy, flow cytometry and Western Blotting for αIIbβ3 expression, and by flow cytometry, spreading assay and cell aggregation for receptor function. Megakaryocytopoiesis and proplatelet formation were investigated by culturing megakaryocytes from peripheral blood CD34+ cells and evaluated by immunofluorescence
Results
The proband was a 2-year-old boy with an ISTH-BAT bleeding score of 8, mild thrombocytopenia (80-120.000/μl) and large platelets. Platelet function tests showed absent αIIbβ3 surface expression and fibrinogen binding and absent aggregation to all stimuli except ristocetin. NGS identified two heterozygous *ITGB3* variants: p.Leu20Arg inherited from the father and p.Asn331Ser inherited from the mother. Family members carrying p.Asn331Ser had macrothrombocytopenia, while those carrying p.Leu20Arg had normal platelets.
In vitro studies with CHO cells revealed distinct effects for the two variants. The novel p.Leu20Arg variant, affecting the β3 signal peptide, led to significantly reduced αIIbβ3 expression as shown by confocal microscopy, flow cytometry, and western blotting. PAC-1 binding, cell spreading, and cell aggregation in the presence of fibrinogen were thus impaired (Fig.1).
The p.Asn331Ser variant resulted in constitutive receptor activation, as assessed by constitutive PAC-1 binding. αIIbβ3 expression on the cell surface was reduced, likely due to receptor internalization. This variant also caused early spreading on fibrinogen and CHO cell aggregation in the presence of fibrinogen under resting conditions.
When both variants were co-expressed, the combined defect was more severe, with further reduced receptor surface expression and significantly impaired cell functions.
Proplatelet formation and spreading on fibrinogen of megakaryocytes cultured from family members carrying p.Asn331Ser were also impaired
Conclusions
Co-inheritance of p.Leu20Arg/p.Asn331Ser led to a phenotype consistent with the patient's severe clinical presentation, resembling type I GT but associated with macrothrombocytopenia. The p.Leu20Arg variant drives receptor degradation, while p.Asn331Ser induces internalization, together causing an additive negative impact on platelet function

Email: *loredana.bury@unipg.it*



A) $\alpha_{IIb}\beta_3$ surface expression assessed with an anti-CD41-FITC antibody by flow cytometry in CHO cells (n=4; *p<0.05 vs WT , ****p<0.0001 vs WT, Kruskal-Wallis test; Box and Whiskerss with min to max).
B) PAC-1 binding to CHO cells assessed by flow cytometry. $\alpha_{IIb}\beta_3$ activation was obtained by incubating cells with 25 mM DTT for 20 min (n=5; *p<0.05 vs WT, **p<0.01 vs WT, ####p<0.0001 vs no DTT; Two way ANOVA with Dunnett's multiple comparison test; Box and Whiskers with min to max).
C) Cell coverage area of CHO cells after 90 min layering on fibrinogen analyzed by fluorescence microscopy. F-actin was stained in red with rhodamine phalloidin. Fiji ImageJ was used to calculate the cell area coverage (n=3; *p<0.05 vs WT, **p<0.01 vs WT, Mann-Whitney test; Box and Whiskers with min to max).
D) Number of cell aggregates analyzed by light microscopy (n=4; ****p<0.0001 vs WT with DTT, § p<0.01 vs WT no DTT; Two way ANOVA with Dunnett's multiple comparison test; Box and Whiskers with min to max).