Functional assays to unravell the pathogenetic role of variants found in GFI1B in piastrinopenic patients

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INTRODUCTION

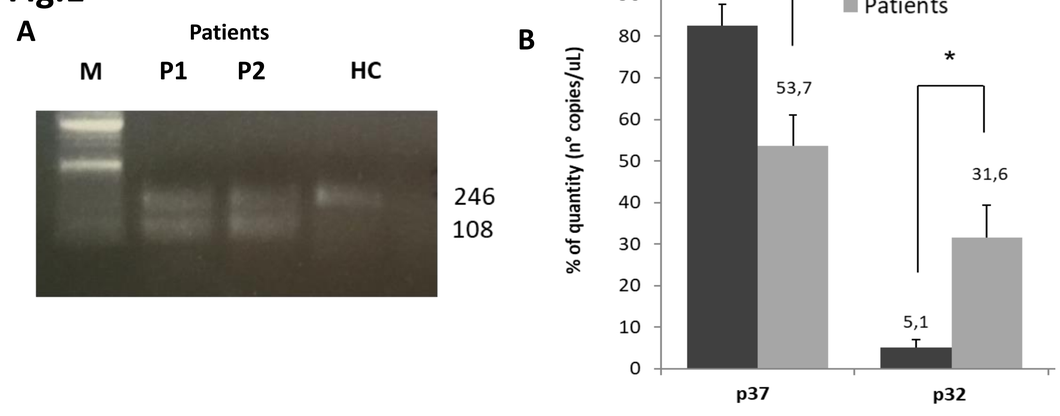
GFI1B is an important transcription factor for megakaryopoiesis, known as causative of a form of Inherited thrombocytopenia (IT) named platelet-type bleeding disorder 17, a heterogeneous disease in which the increased expression of CD34 on platelet surface is the major character in common between patients. NGS analysis of IT patients, allow us to identify five new missense and a splicing variants**.**

AIM

Unravelling the functional impact of nonsense or frameshift variants is usually easy, while assessing the role of missense or splicing variants requires gene-specific functional studies.

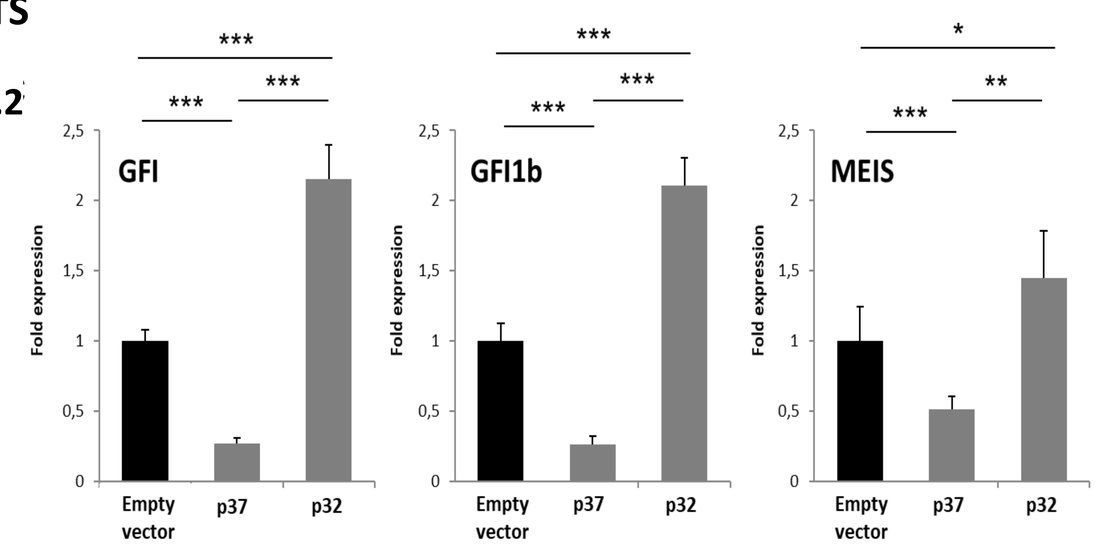
RESULTS

Fig.1



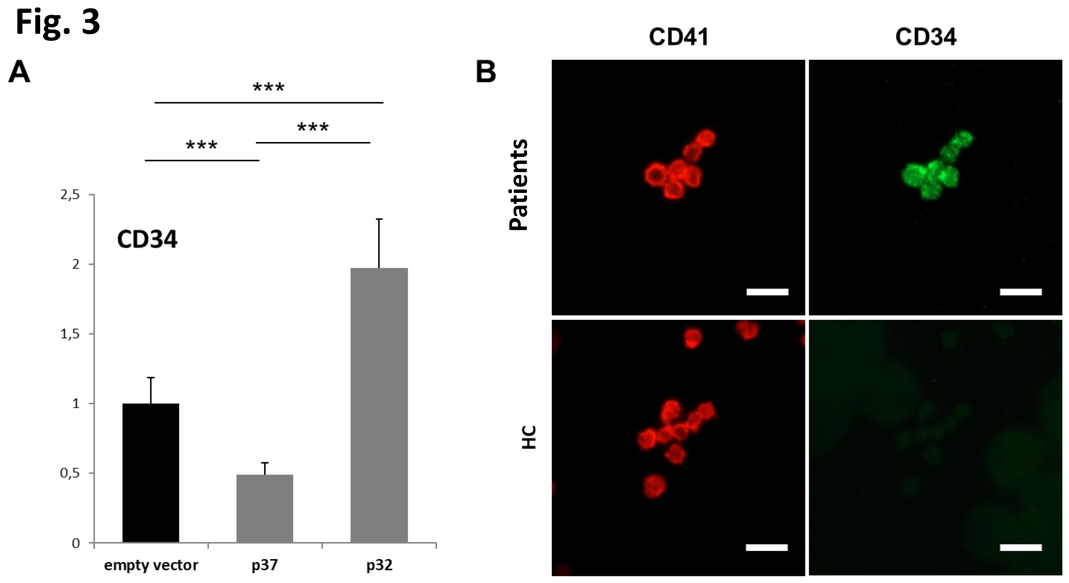
A) RT-PCR performed on patients and healthy control identified the skipping of exon 9 patients P1 and P2 (band of 108 bp, p32 form). **B)** Real Time PCR shows that p32 (with skipping of exon 9) is significantly increased in patients respect to healthy control (*p<0.05), causing an imbalance in p32/p37 ratio.

Fig.2



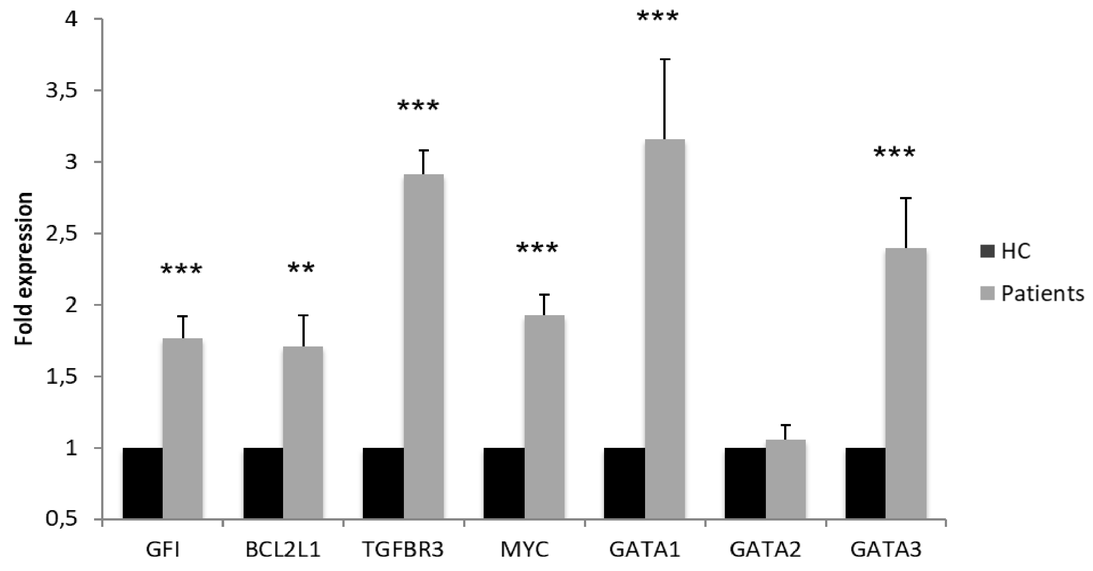
Evaluation of luciferase expression of p37 and p32 isoforms measured on GFI, GFI1B and MEIS promoters in Meg01 cells allows to asses the role of the two isoforms identified. P37 shows a repression activity as expected because GFI1B is a transcription repressor, while p32 form loses the repression activity and instead seems to act as an activator. (***p<0,001)

Fig. 3



A) Since aberrant CD34 expression is the main characteristic of GFI1B patients, we performed luciferase expression of p37 and p32 isoforms of GFI1B also on CD34 promoter after transient transfection in Meg01 cells. Even in this case the p32 form shows a loss of repression activity when compared to the p37 wild type form (***p<0,001). **B**) Moreover, CD34 expression is detected on patient's platelet surface (green labelled) (scale bars=5um).

Fig. 4



p32 form of GFI1B was previously described in literature because is increased in chronic and acute leukaemia. For this reason, we performed Real Time PCR on seven oncogenic GFI1B target genes. All the targets, except for GATA2, show a significant increase in mRNA level (***p<0,001), suggesting that p32 could play a role in haematological malignancies

CONCLUSIONS

Our findings suggest that the splicing variant has probably a role in thrombocytopenia, because shows a dysregulation in its activity. Moreover, the dysregulation of some oncogenic genes suggests that overexpression of p32 could be involved in onset of hematological malignancies, thus making patients more susceptible to develop these diseases. These data demonstrate the fundamental importance of functional analysis in order to evaluate the role of the missense variant identified both for patient management and for a better understanding of the disease.

CONTACT INFORMATIONS

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