A novel splice donor mutation in the thrombopoietin gene leads to exon 2 skipping in a Filipino family with hereditary thrombocythemia

In contrast to the familial predisposition observed in somatically acquired myeloproliferative neoplasms (low penetrance, clonal hematopoiesis), the hereditary thrombocythemias (HT) are characterized by Mendelian inheritance, high penetrance, and polyclonal hematopoiesis, and appear to only affect the megakaryocytic lineage.1 All the molecular alterations identified thus far in patients with HT have involved either THPO (thrombopoietin) or its receptor MPL (myeloproliferative leukemia virus oncogene) genes, with 4 and 3 distinct mutations reported, respectively. The HT-associated THPO mutations were either confirmed or expected to increase the translational efficiency of thrombopoietin without altering the sequence of the mature protein.1 Thrombopoietin, the primary regulator of megakaryopoiesis and platelet production, is produced in the liver, kidney, spleen, and bone marrow.2 Thrombopoietin binds to its receptor and activates the JAK-STAT signaling pathway.3 The presence of multiple upstream AUG codons (uAUG) within the 5′-untranslated region (5′-UTR) precludes efficient translation and prevents harmful overproduction of this potent cytokine.2 We identified a novel point mutation at the splice donor site of THPO intron 2 (position +2) in a Filipino family with HT. Approval was obtained from the Stanford University institutional review board for these studies and informed consent was provided according to the Declaration of Helsinki. The proband and her 2 children manifested with moderate to severe elevations of the serum thrombopoietin levels (measured with the human TPO Quantikine kit, R&D Systems) in the affected family members were significantly higher than in the non-affected family members or healthy controls, while there was no statistically significant difference

References


To the editor:

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between the latter 2 groups (Figure 1C). To examine the functional effects of increased thrombopoietin levels on downstream JAK-STAT signaling, phospho-specific flow cytometry was performed as previously described4 on peripheral blood samples from the family members. As shown in Figure 1D, basal phosphorylated STAT5 (pSTAT5) levels in myeloid progenitors were evaluated and the 95th percentile is presented in the mean with SEM format. An un-paired t test revealed that pSTAT5 levels are significantly higher in the affected group (*P = .024).

Figure 1. THPO mutation inactivates the intron 2 splice donor and correlates with elevation of thrombopoietin level. (A) Pedigree of the 6 family members with serum thrombopoietin concentrations (pg/mL) in the 1st row and platelet counts (×10^3/mm³) in the 2nd row. The proband (II-1) and her 2 children (III-1, III-2) exhibit thrombocytosis and high serum thrombopoietin levels. The proband’s parents (I-1, I-2) and husband (II-2) have normal platelet counts and thrombopoietin levels. (B) A novel heterozygous T>C point mutation at the splice donor site of THPO gene intron 2 was identified through Sanger sequencing in the proband and her children, but not in her parents or husband. (C) The serum thrombopoietin concentrations of family members with the THPO mutation were significantly higher than those family members without the mutation (P < .01), or healthy controls (P < .001). The thrombopoietin levels in the latter 2 groups showed no statistically significant difference. This analysis is performed with 1-way ANOVA followed by the Student-Newman-Keuls multiple comparisons test and the data are presented in the mean with SEM format. D) Basal phosphorylated STAT5 (pSTAT5) levels in CD3+/CD66+/CD14+ myeloid progenitors were evaluated and the 95th percentile is presented in the mean with SEM format. An un-paired t test revealed that pSTAT5 levels are significantly higher in the affected group (*P = .024). (E) Top panel: schematic of the cloned inserts used for exon trapping. These inserts were amplified from patient genomic DNA, cloned into the pCR2.1-TOPO Vector, and then subcloned into the pSPL3b exon trapping vector. Middle panel: expected splicing products from the constructs of the cloned sequence within pSPL3b. In the presence of a mutation in intron 2, exon 2 is expected to be spliced out of the resulting product. Bottom panel: electrophoretic visualization of cDNA-PCR products amplified from the constructs after transfection into COS-7 cells. RNA was extracted and reverse transcribed to cDNA 48 hours after the transfection of the pSPL3b-Insert construct into COS-7 cells. PCR was performed using primers SD6 and SA2, and products were resolved on a 2% agarose gel. Splicing of the vector alone yields a 261bp fragment resulting from the flanking vector exons. Splicing of the wild-type Insert 1 and Insert 2 constructs results in 547bp and 634 bp fragments, respectively. The mutant splice products display fragments that are 158bp shorter, indicating complete splicing-out of exon 2.
Increased coagulation factor VIII activity in patients with familial hypercholesterolemia

Coagulation factor VIII (FVIII) plays a crucial role in the coagulation cascade, but the factors, environmental or hereditary, determining its levels, are hitherto largely unknown. Murine in vivo data and genetic association studies have recently suggested a role for the low-density lipoprotein receptor (LDL-receptor) in the regulation of this coagulation factor.1,2 Martinelli and colleagues have suggested to study patients with familial hypercholesterolemia (FH) to address the consequences of low expression levels of LDL-receptor in modulating FVIII levels.3 In the current study, we did determine FVIII levels in individuals that underwent cascade screening for genetic FH, hypothesizing that patients who lack functional LDL-receptors would have higher FVIII levels than their unaffected relatives.

The study population derived from a cross-sectional study described in detail before.3 In short, 421 individuals were invited within 18 months after genetic testing for FH and both non-affected relatives and FH patients were eligible. The study was approved by the local ethics committee and all participants gave written informed consent. For the current study, we had to exclude 156 individuals: 122 subjects were on lipid-lowering treatment at the time of study visit; 30 patients were identified with a pathogenic mutation in the LDL-receptor in modulating FVIII levels.1 In the current study, we demonstrated that patients with heterozygous FH had on average a significant 9% higher FVIII level than unaffected relatives. This difference remained after adjustments for family ties (mean ± SE 102.8 ± 2.9 versus 95.4 ± 2.1, P = .037) and age (103.5 ± 2.9 versus 94.7 ± 2.2, P = .019). The association between LDLR-mutation and high FVIII levels remained statistically significant after additional adjustment for VWF, CRP and LDL-cholesterol (data not shown).

We demonstrated that patients with heterozygous FH had on average a significant 9% higher FVIII level than unaffected relatives. This finding confirms the hypothesis derived from previous findings, suggesting that the LDLR might have a suppressing role on FVIII levels.

Strength of the current study is that participants were recruited from families participating in genetic cascade screening, so in essence free from referral bias. A potential limitation is that a myriad of different LDLR mutations were present in our study population.3,5 If LDLR activity is indeed a determinant of FVIII
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