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is critical for megakaryocyte development. Therefore, when the nonaffected allele is expressed, normal megakaryocyte development occurs; but when the deleted allele is (the one expected to be) expressed, megakaryocyte development is restricted, giving rise to a subpopulation of small megakaryocytes.⁶ Recently, the role of *FLII* and other ETS transcription factors in megakaryopoiesis and platelet function have been underscored by reports of enrichment of *FLII* mutations in individuals with functional platelet defects as well as the recent discovery that mutations in the gene *ETV6* also cause thrombocytopenia.⁷⁻⁹

In their Brief Report, Stevenson et al describe a family with 2 siblings, affected by a lifelong history of moderate thrombocytopenia and excessive bleeding with seemingly unaffected parents. The patients' platelets exhibit abnormal response to traditional agonists such as collagen and adenosine diphosphate, and platelet electron micrographs show large, fused electron-dense α -granules characteristic of Paris-Trousseau thrombocytopenia (see figure). Sequencing of *FLII* revealed a homozygous missense mutation at position 970 (c.970C>T) that predicts an arginine to tryptophan substitution in the conserved DNA-binding domain of *FLII*.¹

Functional analysis of the c.970C>T mutation by a luciferase reporter assay demonstrated decreased transcriptional activity of known gene targets of *FLII*, including *GP6*, *GP9*, and *ITGA2B*, with a concomitant reduction of the respective protein expression in the cell lines as well as in platelets of affected individuals, strongly indicating a role of the *FLII* mutation in the platelet phenotype. As the parents do not show a platelet defect despite being heterozygotes for the c.970C>T mutation, the mechanisms that govern the autosomal dominant thrombocytopenia associated with *FLII* hemizygous deletions are still unclear.

Overall, despite robust cumulative genetic and biological evidence pointing to the role of *FLII* in Paris-Trousseau thrombocytopenia, the involvement of other genes in the 11q deletion syndrome could not be ruled out. The report by Stevenson et al, in which a point mutation in *FLII* is associated with the same phenotype, adds strong evidence to the case for *FLII* as the main driver of the Paris-Trousseau thrombocytopenia.

● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Stevenson et al, page 2027

Paris-Trousseau: evidence keeps pointing to *FLII*

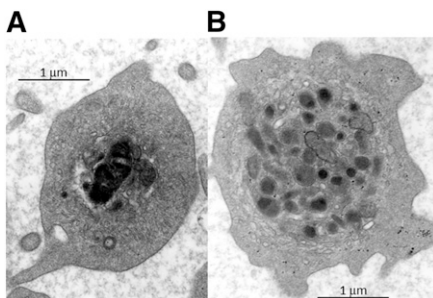
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In this issue of *Blood*, Stevenson et al describe a family with a homozygous missense mutation in *FLII* that is associated with a platelet phenotype identical to the one observed in Paris-Trousseau syndrome, supporting existing evidence that *FLII* is directly involved in the mechanism of thrombocytopenia observed in this disease.¹

Patients with a terminal deletion of the long arm of chromosome 11 exhibit several developmental abnormalities and distinctive facial features. This rare disorder is known as Jacobsen syndrome. A large majority of these individuals (>90%) have a bleeding diathesis called Paris-Trousseau thrombocytopenia characterized by autosomal dominant thrombocytopenia and a subpopulation of platelets that exhibit abnormal responses to thrombin and contain giant α -granules.² The

thrombocytopenia can be severe but often resolves during adolescence. The bleeding phenotype is variable with reports of excessive bleeding even after normalization of the platelet counts, indicating an intrinsic platelet defect.³ One of the characteristic findings in the bone marrow of patients with this disorder is the presence of 2 populations of megakaryocytes: 1 population of normal appearing megakaryocytes and the other one represented by micromegakaryocytes.

The 11q deletions can span up to 16 megabases and 300 genes with several of them being potential candidates for the platelet phenotype. Among these, *FLII* (a member of the ETS [E-twenty-six] family of transcription factors), is a natural candidate because it has been identified as a key regulator of megakaryopoiesis and its hemizygous deficiency has been previously associated with defective megakaryocyte development in a murine model.^{4,5} Additionally, all individuals with Paris-Trousseau thrombocytopenia have deletions that are centromeric to *FLII*. Interestingly, it has been proposed that transient monoallelic expression of *FLII*



Electron micrographs of platelets from a patient homozygous for the *FLII* c.970C>T mutation show large electron-dense fused α -granules (A) when compared with a normal platelet electron micrograph (B). See Figure 1E in the article by Stevenson et al that begins on page 2027.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Kautz et al, page 2031

Erythroferrone: the missing link in β -thalassemia?

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In this issue of *Blood*, Kautz et al¹ show that the ablation of the erythroid-derived factor erythroferrone (ERFE), which has been shown to be highly expressed in β -thalassemic mice,² restores hepcidin levels and corrects iron overload. However, correction of hepcidin levels in those mice does not improve anemia of β -thalassemia.

β -Thalassemias are monogenic diseases characterized by the lack or reduction of the hemoglobin β -globin chain expression resulting in an increase of the α/β globin ratio. The excess free α chains aggregate and precipitate in erythroblasts, leading to damage of cell membranes and reactive oxygen species (ROS) generation, leading to ineffective erythropoiesis. Ineffective erythropoiesis is characterized by an expansion of immature erythroblasts associated with apoptosis of mature erythroblasts at the polychromatophilic stage, leading to a major reduction of red cell production.^{3,4} In addition, ineffective erythropoiesis of β -thalassemia is associated with iron overload.³ Depending on the specific α/β -globin ratio and capacity for fetal hemoglobin synthesis, patients can present at various ends of the phenotype spectrum of

biological and clinical severity. Patients with the most severe forms (β -thalassemia major) require chronic red blood cell transfusion for survival and iron chelation to prevent increased plasma iron and formation of non-transferrin-bound iron (NTBI) with its related organ damage (eg, liver, heart, and/or endocrine organs).⁴

Patients associated with a milder phenotype (β -thalassemia intermedia or non-transfusion-dependent thalassemia) may need only sporadic blood transfusions. However, because of ineffective erythropoiesis, these patients exhibit increased iron absorption and NTBI, leading to severe iron overload, its clinical manifestations, and eventually death. In addition, iron overload may further aggravate ineffective erythropoiesis by stimulating erythroblast ROS production, which increases

the α/β -globin chains imbalance. ROS may also stimulate growth differentiation factor 11 (GDF11) expression, a novel actor in ineffective erythropoiesis that causes erythroid expansion of immature erythroblasts and inhibition of end-stage erythroid maturation.⁵ For the last decade, investigators have focused on understanding the mechanisms underlying iron overload in ineffective erythropoiesis, hypothesizing that correction of ineffective erythropoiesis would significantly reduce iron overload and improve anemia, ultimately increasing overall survival of patients with β -thalassemia.

Hepcidin, a small peptide mainly produced by the liver, is absolutely required for the maintenance of systemic iron homeostasis in basal conditions.⁶ Hepcidin controls serum iron levels by binding to ferroportin (FPN), the only known iron exporter, and inducing its degradation. Low hepcidin stabilizes FPN at the cellular membrane, promoting dietary iron absorption in the duodenum, increasing the release of iron from macrophages following erythrophagocytosis, and enabling iron mobilization from hepatocytes. Likewise, hepcidin is suppressed in conditions associated with accelerated erythropoiesis (eg, anemia due to bleeding, hemolysis, or iron deficiency) and ineffective erythropoiesis (eg, β -thalassemia).

Two members of the transforming growth factor- β superfamily, GDF15 and twisted gastrulation,^{7,8} have been proposed as pathologic suppressors of hepcidin in ineffective erythropoiesis, but several experimental and in vivo data in human favor at most a minor role for all these factors in iron overload in ineffective erythropoiesis. In contrast, the Ganz laboratory has shown that ERFE, a member of the C1q-tumor necrosis factor-related family of proteins, is the major negative regulator of hepcidin in conditions of stress or ineffective erythropoiesis.² ERFE is produced by erythroid precursors in the bone marrow on erythropoietin (EPO) stimulation and represses liver hepcidin production by a still unknown mechanism.

ERFE is probably not the sole erythroid regulator of hepcidin because adult Erfe-deficient mice had normal hematologic and iron parameters. Kautz et al¹ elegantly show that ERFE messenger RNA levels were remarkably elevated in the bone marrow and the spleen of a mouse model of β -thalassemia intermedia and that ablation of ERFE in thalassemic mice restores hepcidin levels to normal and