of soluble receptor generation has been shownalready for the generation of soluble cytokine receptors.

Biological consequences of increased sEPCR (and/or the resulting decreased membrane EPCR density) may be related to increased procoagulant activity (eg, higher risk of thrombosis). There may also be an impact on the cytoprotective activity of APC, which depends on membrane EPCR and PAR-1. For both functions of PC/APC, the A3 haplotype would be unfavorable. However, there may also be beneficial effects of decreased EPCR receptor density on the cell surface (and thus of the A3 haplotype), since it also has been shown that APC mediates breast cancer cell migration through interactions with EPCR and PAR-1.[5]

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

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HEMATOPOIESIS & STEM CELLS

Comment on Ghevaert et al, page 3407

Sizing up platelet defects

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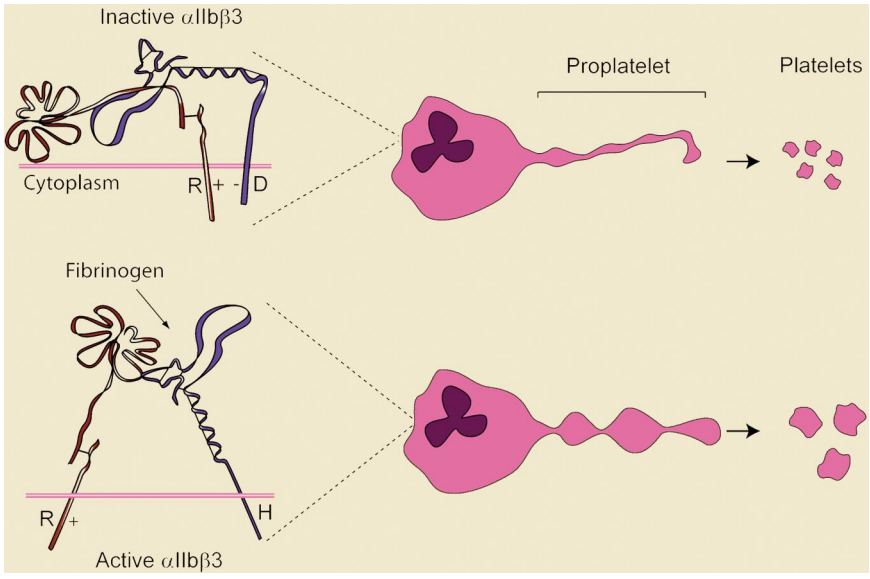
In this issue of *Blood*, Ghevaert and colleagues show that a mutation in the cytoplasmic domain of integrin β_3 is associated with dominantly inherited macrothrombocytopenia. This mutation is predicted to disrupt a conserved salt bridge with integrin α_{IIb}, and constitutively activates the integrin.

Large platelets are often encountered in the evaluation of thrombocytopenias, but most often these are acquired conditions. A rather long list of inherited thrombocytopenias is now appreciated.[1] Among the macrothrombocytopenias, the most commonly responsible genetic variations are heterozygosity for mutations in platelet glycoprotein Ibα and the Q43P polymorphism of β1 tubulin. The molecular regulation of platelet size is poorly understood, but circumstantial evidence supports a role for the platelet cytoskeleton and microtubular system. The lion's share of this evidence stems from the study of genetic defects in inherited disorders of platelet size. Although these disorders are quite uncommon, an understanding of the molecular basis of platelet size is important because subjects with abnormally high platelet volumes have enhanced platelet reactivity and are at risk for recurrent ischemic coronary syndromes.[2]

Ghevaert and colleagues performed a thorough series of studies characterizing a pedigree for macrothrombocytopenia, with the mean platelet volume of the propositus measuring a whopping 17 fL. All affected individuals had heterozygous mutations: P53L in the gene encoding GPIbα (*GP1BA*), and D723H in the gene encoding integrin β3 (*INTB3*). At least 4 syndromes (Bernard-Soulier, DiGeorge, benign Mediterranean macrothrombocytopenia, and platelet-type von Willebrand disease) are associated with macrothrombocytopenia and *GP1BA* mutations. However, convincing data were provided to suggest that the *GP1BA* P53L mutation was not responsible for the phenotype in this pedigree, and molecular modeling predicted that the *GP1BA* P53L substitution would not affect von Willebrand factor binding. Platelets from subjects with the *INTB3* D723H mutation showed spontaneous $\alpha_{IIb}\beta_3$ activation, and CHO cells expressing $\alpha_{IIb}\beta_3$-D723H displayed enhanced binding to immobilized fibrinogen. Intriguingly, in vitro–differentiated megakaryocytes with the β_3-D723H mutation exhibited larger proplatelet buds than the wild type.

It was not long ago that molecular genetic studies were rather primitive compared with the thorough multidisciplinary approaches used by Ghevaert and colleagues to characterize this pedigree. Several novel findings are presented and questions raised. First,



Model summarizing how integrin activation state may regulate platelet size. The top panel indicates how the salt bridge between wild-type αIIb-R995$^+$ and β3-D723$^-$ constrains the integrin in an inactive conformation. The β3-D723H mutant disrupts this linkage and activates the receptor. Abnormally large platelets result via an unknown mechanism.

integrin mutations should now be added to the list of causes of macrothrombocytopenia. Second, the naturally occurring mutation in β_3 supports the existence and function of the conserved intracellular salt bridge between α_{IIb}-R995 and β_3-D723 that was proposed using elegant in vitro charge-reversal mutation studies (see figure).[3] Third, although the interaction of $\alpha_{IIb}\beta_3$ with fibrinogen stimulates proplatelet development,[4] a mechanism that integrates the induction of proplatelet formation due to the β3-D723H mutation with the formation of large platelets remains to be established. Perhaps this phenotype results from a simple physical effect whereby increased proplatelet adhesion to bone marrow sinusoidal fibrinogen delays platelet release while size continues to increase. Or perhaps, since integrin binding to extracellular matrix induces gene transcription,[5] the enhanced adhesion of β3-D723H induces expression of genes regulating platelet size or favors cross-talk with GPIbα, tubulin, or other cytoskeletal proteins.

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

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NEOPLASIA

Comment on Dulucq et al, page 3692

One man's dose, another man's poison

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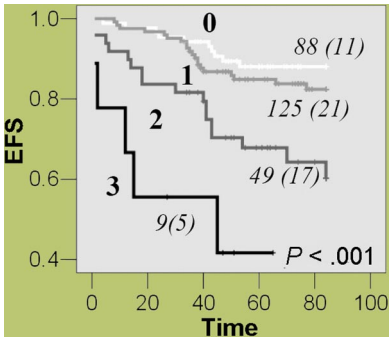
In this issue of *Blood*, Dulucq and colleagues report that genetic polymorphisms in the promoter of dihydrofolate reductase (*DHFR*) are linked to expression and outcome in childhood acute lymphoblastic leukemia (ALL).

The survival of children with ALL has improved steadily over the past 5 decades, primarily as a result of optimizing the use of recognized antileukemia drugs and stratifying patients at greatest risk of relapse using age, white cell count, immunophenotype, karyotype, and response to therapy.[1] Patients with specific polymorphisms in genes responsible for metabolizing leukemia drugs can also have an increased risk of relapse or excessive treatment toxicity.[1]



Combined effect of multiple event-predisposing genotypes—DHFR (haplotype *1), thymidylate synthase (TS) 3R3R, and cyclin D1 (CCND1) AA870—on event-free survival in childhood ALL patients. For each group of patients with 0, 1, 2, or 3 of the specified genotypes, the number of patients (and the number of events) are given.

Methotrexate is a key antileukemia drug whose principal effect is the inhibition of DHFR, which impairs purine and thymidine synthesis and ultimately causes cell death.[2] Dulucq and colleagues investigated whether promoter polymorphisms in *DHFR* affect outcome in childhood ALL. Genotyping of 48 control subjects revealed a total of 15 polymorphisms, including 3 single-nucleotide polymorphisms (SNPs)—C-1610G/T, C-680A, and A-317G—that could be used to define 5 main haplotypes. The frequency, prognostic relevance, and biological effect of these genotypes and haplotypes were ascertained in 277 children in remission from ALL. Patients homozygous for the A-317 or C-1610 alleles or who were carriers of the associated haplotype (*1) had a 70% to 80% increased risk of an adverse event compared with other patients, and this effect was independent of traditional risk factors. The authors postulate that the higher levels of DHFR mRNA associated with these genotypes were responsible for adverse patient outcome. Acknowledging the fact that numerous genes are involved in methotrexate metabolism, they investigated the effect of multiple event-predisposing genotypes. Event-free survival was inversely proportional to the number of risk genotypes—*DHFR* haplotype *1, thymidylate synthase 3R3R, and cyclin D1 AA870—and patients with all 3 were 8 times more likely to suffer an event compared with those without any of these genotypes (see figure).

This study contributes to the growing body of literature advocating a role for pharmacogenetic risk factors in the management of childhood ALL.[1] Although this study has a number of strong points, there are also some important limitations. Key strengths include establishing the link between genotype and mRNA levels, providing mechanistic evidence for the association, and providing a clear rationale for the observed treatment response. When polymorphisms affecting other genes in the same pathway were taken into account, the effect increased, underlining the importance of methotrexate response in treating ALL. Although these results need independent confirmation, it is interesting to note that patients in this study were treated on 4 consecutive protocols with a consistent methotrexate dose. One limitation of the study is its lack of cytogenetic data. Only one cytogenetic subgroup, high hyperdiploidy, was considered in the multivariate analysis despite numerous others being highly relevant to prognosis.[1] There is also evidence to suggest that acquired genetic abnormalities can alter germline phenotype and hence modify leukemic blast response to a drug.[3]

Children diagnosed with ALL today have an excellent chance of being cured,[1] and new protocols are focusing on the reduction of treatment intensity. Therefore, pharmacogenetic risk factors will become increasingly important. Future studies will