transposon systems such as PB. PB may offer advantages such as a larger cargo load (>14 kb, allowing the inclusion of more genes or larger regulatory elements), different insertion preferences, and the exclusive use of TTAA target sequence.1,2 For some applications, the PB transposition offers an additional advantage: the integrated transposon can be precisely excised by re-expression of PB transposase transiently as shown by several groups in diverse systems. Although the current PB system is inferior for HSPCs to the SB system using the superpac SB100X, I predict that the PB transposase will be improved significantly soon. Together, these transposon systems offer hopes to achieve virus-free, long-term gene transfer and expression in HSPCs and other cell types for various forms of gene therapy.

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

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In this issue of Blood, Jones and colleagues use a genotype-phenotype approach to identify novel quantitative trait loci associated with platelet signaling pathways.

Interindividual variation in platelet reactivity likely contributes to occlusion of coronary or cerebral arteries upon atherosclerotic plaque rupture in some individuals, whereas other individuals repair such lesions without occluding the vessel. It is expected that few (or fewer) genes are involved in any one of the intermediate traits (eg, platelet reactivity) compared with a larger spectrum of genes that contribute to the final manifestation of the clinical disease (eg, pathologic arterial thrombosis; see figure). This is the rationale supporting genetic association studies of intermediate phenotypes because the power to detect gene associations is enhanced when the potential number of genes responsible for the phenotype is reduced or refined. Thus, this improves the fraction of variance explained by any single factor or gene.1 Whereas it is relatively easy to measure substances (such as blood lipids) in a high-throughput manner, it is vastly more complex to assess ex vivo functions of living tissues. Thus, for platelet genomic research, there is a “catch–22”: there is value in using ex vivo platelet function as an intermediate end point, and yet it is challenging to study the thousands of samples typically required to achieve the analytic power needed to satisfy accepted statistical thresholds.

In this issue of Blood, Jones et al demonstrate how moderate sample sizes—in the few hundreds—have value as a screening tool when complemented by “biologic filters” that help prioritize gene lists and, importantly, “wet bench” studies confirming the computational associations.2 The authors developed a list of 97 genes believed to be important in the function of platelets and other hematopoietic cells. These 97 genes were resequenced in 48 European samples, novel SNPs were identified with low minor allele frequencies, and 1327 SNPs were selected that tagged most of the genetic variation in these genes. DNA from 500 healthy subjects with known platelet responses to cross-linked collagen-related peptide (CRP) and ADP3 was genotyped for these 1327 SNPs. Statistical analyses revealed 17 novel associations with platelet function, including genes encoding cell surface receptors (CD36, GP1b, ITGAV2, PEAR1, and P2Y12), kinases (JAK2, MAPK2, MAPK2K4, MAPK14) and other signaling molecules (GNAZ, VAV3, ITTPR, FCERG1). The associations presented in Table 1 of the article and other supplemental materials will be of great interest to the platelet biology community.

The authors focused on SNPs in 3 genes of interest, PEAR1, VAV3, and ITTPR, for validation at the protein and physiologic

Figure 1. The utility of intermediate traits as end points. This greatly simplified schematic assumes there are 10 genes contributing equally to the clinical end point (ie, 10% each, faint dashed lines). Another level of complexity to the pathophysiology is superimposed by including 5 intermediate traits, each contributing 20% to the clinical outcome. In this example, genes 1 and 2 have a much larger effect on platelet reactivity (50%) than on the clinical end point (10%). Of course, environmental factors also play a major role in these end points (not shown).
levels. Genetic variation in PEAR1 has previously been associated with collagen-induced platelet aggregation, and Jones et al. found that PEAR1 SNPs showed the strongest of all associations with the platelet response to CRP, as well as strong associations with ADP-induced fibrinogen binding. PEAR1 genotypes were associated with PEAR1 levels (the minor alleles showing increased expression). A minor allele of a VAV3 SNP was associated with increased protein levels, and ITPR1 genotypes were associated with platelet calcium flux in response to ADP.

The devil is in the phenotypic details of genotype–phenotype association studies. Over the past few years, a number of groups have developed different cohorts to systematically address the complex relationship between genetic variation and platelet phenotypes. Of note, the Wellcome Trust platelet assays were supplemented with aspirin, hirudin, and, for CRP-stimulated samples, apyrase, such that these platelet phenotypes are expected to display maximum sensitivity to GPVI-mediated platelet activation and be insensitive to the amplifying effects of thromboxane A2 and ADP. This likely contributes to the large effect size seen for GP6 SNPs on CRP-induced platelet reactivity. This aspect of the assay design is neither better nor worse than designs that lack such inhibitors—but is expected to uncover some gene associations different from those found by genomic studies using alternative assay designs. This study comprised healthy subjects of Northern European ancestry, and cannot be extrapolated to patients or different ethnic groups. It should be noted that the follow-up experiments with PEAR1, VAV3, and ITPR1 SNPs also defined associations and not causality. Genome-wide association studies (GWASs) are currently a “topical” genomics technology and have successfully identified common genetic variants associated with the risk of numerous common diseases. But the great majority of variants have had odds ratios less than 1.5, and many thousands of subjects have been required to achieve statistical significance. Although unbiased, GWASs identify genomic regions, not genes themselves, and the findings usually lack biologic context. Therefore, well-conducted, candidate gene studies such as the one by Jones et al still have a place in the armamentarium of platelet genomic studies.

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

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TRANSPLANTATION

Comment on Sarzotti-Kelsoe et al, page 1445

TRECing long-term success in SCID

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In this issue of Blood, Sarzotti-Kelsoe and colleagues provide strong evidence that hematopoietic stem cell transplantation in primary T-cell deficiencies provides long-term maintenance of thymopoiesis and T-cell function.

Allogeneic hematopoietic stem cell transplantation (HSCT) has become the established therapy for primary T-cell immune deficiencies, but concerns have remained about the long-term fate of thymopoiesis, particularly in patients in whom donor cells predominate only in the T-cell compartment. In this definitive study of a large cohort of recipients followed for as long as 25 years, Sarzotti-Kelsoe et al provide evidence that these transplants maintain T-cell function, thymic output, and T-cell–receptor repertoire diversity. Severe combined immune deficiencies (SCIDs) are rare disorders that affect T-cell and, sometimes, B-cell and NK-cell production. They cause an inability to mount antigen–specific responses. SCID infants are highly susceptible to recurrent viral infections, and without treatment, rarely survive the first year of life. Marking 40 years since the first HSCT therapy in SCID children in 1968, a 2008 North American workshop noted that the key question was the extent and durability of T-, B-, and NK-cell reconstitution and function. Marking the same anniversary, several European centers have recently reviewed their experiences regarding long-term survival and T-cell function in SCID recipients.

These reports complement the Sarzotti-Kelsoe report in demonstrating the broad success of hematopoietic cell transplantation (HCT) in treating SCID immune deficiency; the expansion of the donor pool to include HLA-matched—related, haploidentical—related, and HLA—matched unrelated donors; and the long-term stability of T-cell repopulation in SCID recipients (see table).

The Sarzotti-Kelsoe study is remarkable for its extensive, sophisticated analyses of thymic productivity and T-cell repertoire. The rearrangement of T-cell receptor (TCR) genes during thymopoiesis was assessed. Rearrangement of V(D)J segments of the TCR α and β chains occurs by the excision of intervening sequences as episomal DNA. The resultant T-cell receptor rearrangement excision circles (TRECs) remain in T cells, but do not replicate. The new appearance of TREC-bearing cells in the periphery is therefore evidence of thymic maturation of T cells after HSCT. Because the frequency of TRECs is reduced by activation-induced expansion, the maintenance of a high frequency of TREC is evidence of continued thymopoiesis. Sarzotti-Kelsoe et al assessed TREC frequencies in serial patient samples collected from 1 to 25 years after transplantation, establishing the durable maintenance of high TREC levels after SCID transplantation.

The main source of diversity of TCR occurs during the rejoining of the V(D)J genes, by the random insertion of nucleotides coding for 6 to 14 amino acids. In newly generated naïve T cells, the relative frequencies of insertions accordingly follow a Gaussian distribution, and expansion of activated T cells results in a skewing of the repertoire and a departure...
Platelet genomics beats the catch-22

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