

# inside **blood** commentary

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## ● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Simeoni et al, page 2791, and Stritt et al, page 2903

# Platelet disorders: the next generation is in

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In this issue of *Blood*, Simeoni et al describe exciting results using a high-throughput sequencing (HTS) platform with 63 targeted genes in patients with heritable bleeding and thrombotic disorders, and Stritt et al advance a diaphanous-related formin 1 (*DIAPH1*) variant as a cause of inherited macrothrombocytopenia (MTP) and hearing loss.<sup>1,2</sup>

**T**hese studies describe elegant genomic approaches driven by HTS (also called next-generation sequencing) in unraveling the genetic abnormalities in patients with bleeding and thrombotic disorders. Each study tells an important story about the means as well as the end, establishing the disease-causing variants. They attest to the power of the HTS technology, advances in bioinformatics, large databases, and application of human phenotype ontology (HPO)—term coding,<sup>3</sup> and of collaborative groups so essential to make rapid inroads.

In the first study, Simeoni et al report the results from the ThromboGenomics Consortium encompassing investigators from across 13 countries, where a targeted panel of 63 genes was assessed in a heterogeneous group of patients with bleeding and platelet disorders (BPD) with known and unknown diagnosis. Included were patients with abnormal platelet count, volume, morphology, or function, or a tendency to bleed abnormally on a genetic basis, along with patients with an abnormal tendency for thrombosis. The HTS platform was designed to detect variants in the exonic fraction of 63 BPD genes, and many of their introns and untranslated regions. Automated variant filtering procedures and HPO

coding-based prioritization of candidate variants were applied.

The authors sequenced 300 samples (260 unrelated subjects) from 4 subject groups: the “known” group (n = 159) with diagnostic laboratory abnormalities and previously established pathogenic genetic variants; the “suspected” group (n = 61) with phenotypes that strongly indicated a particular disorder on the basis of laboratory abnormalities but without knowledge of causal variants; the “uncertain” group (n = 76) with phenotypes that could not be matched to any known BPD because the laboratory assays were either normal or not diagnostic of an established disorder; and 4 unaffected relatives.

In the “known” group, the ThromboGenomics platform correctly called the pathogenic variants in all 159 samples (with 145 causal variants), with an impressive sensitivity of 100%. In the “suspected” group, in 56 of the 61 subjects a pathogenic or likely pathogenic variant was identified, including 5 samples where the prior Sanger sequencing was unrevealing. These patients had entities such as Glanzmann thrombasthenia (4 patients), Bernard-Soulier syndrome (1), Hermansky-Pudlak syndrome (9), May-Hegglin disorder (4), or a plasma factor

deficiency (26), with clinical and laboratory findings that suggested the diagnosis and candidate gene(s).

The highly “uncertain” group consisted mainly of patients with bleeding symptoms but with normal laboratory tests, storage pool disorder (presumably indicated by abnormalities on platelet function testing), or patients with a thrombotic event and low plasma protein S. In these 76 subjects (62 unrelated), pathogenic or likely pathogenic variants were detected in only 8 cases (sensitivity, 10.5%). Thus, in these patients without a clear phenotypic lead, the yield from interrogating genes previously implicated in BPD patients was low. These data indicate that there are many more causal genes involved in BPD patients than identified to date and that the unknown causal genes/variants may outnumber those known. The unknown variants may be in other genes or in the unexplored regulatory regions. Incorporating into the ThromboGenomics platform additional genes that become linked to BPD disorders may make it a stronger test. Also, the platform does not detect inversions, and these are known to occur in some BPD patients (such as hemophilia A). Whole exome sequencing, as applied in identifying the mutation in *DIAPH1*<sup>2</sup> and other genes,<sup>4-8</sup> may provide a solution. Exomes account for ~2% of the genome; whole genome sequencing may be required in some. But this imposes additional requirements, including cost. The ThromboGenomics Consortium includes patients with bleeding symptoms (platelet and coagulation defects) and thrombotic phenotypes. Eventually, the effectiveness of the platform in each group needs to be established and may be different.

Overall, the studies of Simeoni et al are an impressive validation of a targeted approach using HTS and HPO coding, particularly in BPD patients with suspected etiology. Its use in the wider population of BPD patients may depend on the success rate in further studies in the “uncertain” group of patients, with additional

genes incorporated into the platform. Patients with platelet function defects and a normal platelet count but without clues on evaluation to known entities are of specific interest. In such patients, the defects are largely unknown.

In the second study, Stritt et al combined exome sequencing, HPO coding, and phenotype similarity regression to identify a novel heterozygous variant in *DIAPH1* (p.R1213\*) in 2 unrelated pedigrees with autosomal dominant MTP and early onset sensorineural hearing loss. Data were analyzed from 702 index BPD cases with unknown genetic mechanisms from the Biomedical Research Centres/Units Inherited Diseases Genetic Evaluation–BPD (BRIDGE–BPD) study<sup>3</sup> and 3453 control subjects. A total of 1073 genes had a rare variant in at least 2 cases and predicted to impact gene translation. The *DIAPH1* variant was not found within the 61 486 exomes in the Exome Aggregation Consortium database. Six affected pedigree members but not 3 asymptomatic members had the variant.

*DIAPH1* encodes the protein that regulates cytoskeletal processes, such as actin assembly and microtubule stability. The *DIAPH1* variant predicts for truncation in the *DIAPH1* diaphanous autoregulatory domain. The authors demonstrate reduced proplatelet formation from megakaryocytes and cytoskeletal alterations consistent with constitutive *DIAPH1* activation, and advance a causal link between the *DIAPH1* variant and MTP and hearing loss. To date, mutations in *MYH9* have been associated with MTP and sensorineural hearing loss. This report extends *DIAPH1* mutations to this constellation, although there were differences. Neutrophil cytoplasmic inclusions and renal failure noted in *MYH9* variants were not observed.

These 2 studies in this issue of *Blood* make important contributions to the field of platelet disorders. Since the advent of HTS, these and other studies<sup>4–8</sup> promise exciting times ahead in the quest to decode the secrets of nature underlying human bleeding disorders.

*Conflict-of-interest disclosure:* The authors declare no competing financial interests. ■

## REFERENCES

1. Simeoni I, Stephens JC, Hu F, et al. A high-throughput sequencing test for diagnosing inherited bleeding, thrombotic, and platelet disorders. *Blood*. 2016; 127(23):2791–2803.
2. Stritt S, Nürden P, Turro E, et al. A gain-of-function variant in *DIAPH1* causes dominant macrothrombocytopenia and hearing loss. *Blood*. 2016;127(23):2903–2914.

3. Westbury SK, Turro E, Greene D, et al; BRIDGE–BPD Consortium. Human phenotype ontology annotation and cluster analysis to unravel genetic defects in 707 cases with unexplained bleeding and platelet disorders. *Genome Med*. 2015;7(1):36.

4. Lentaigne C, Freson K, Laffan MA, Turro E, Ouwehand WH, on behalf of the BRIDGE–BPD Consortium and the ThromboGenomics Consortium. Inherited platelet disorders: toward DNA-based diagnosis. *Blood*. 2016;127(23):2814–2823.

5. Turro E, Greene D, Wijgaerts A, et al; BRIDGE–BPD Consortium. A dominant gain-of-function mutation in universal tyrosine kinase SRC causes thrombocytopenia, myelofibrosis, bleeding, and bone pathologies. *Sci Transl Med*. 2016;8(328):328ra30.

6. Leo VC, Morgan NV, Bem D, et al; UK GAPP Study Group. Use of next-generation sequencing and candidate

gene analysis to identify underlying defects in patients with inherited platelet function disorders. *J Thromb Haemost*. 2015;13(4):643–650.

7. Watson SP, Lowe GC, Lordkipanidzé M, Morgan NV; GAPP Consortium. Genotyping and phenotyping of platelet function disorders [published correction appears in *J Thromb Haemost*. 2013;11(9):1790]. *J Thromb Haemost*. 2013;11(suppl 1):351–363.

8. Albers CA, Cvejic A, Favier R, et al. Exome sequencing identifies NBEAL2 as the causative gene for gray platelet syndrome. *Nat Genet*. 2011;43(8):735–737.

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## ● ● ● HEMATOPOIESIS AND STEM CELLS

Comment on Vukovic et al, page 2841

# The identity crisis of Hif-1 $\alpha$ in HSC biology

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In this issue of *Blood*, Vukovic et al provide compelling data that hematopoietic stem cells (HSCs) do not require the transcription factor hypoxia-inducible factor 1 $\alpha$  (Hif-1 $\alpha$ ) to duplicate themselves (ie, self-renew), reconstitute long-term hematopoiesis, or sustain hematopoiesis following injury.<sup>1</sup> These observations affect hematology because they challenge previous notions regarding the role of Hif-1 $\alpha$  in HSC biology.<sup>2</sup>

**A**dult HSCs are multipotent progenitors that can sustain themselves as well as give rise to the various blood cell lineages for the lifetime of an organism.<sup>3</sup> Mammalian HSCs reside in a specialized microenvironment within the bone marrow (BM) called the HSC niche. HSC behaviors such as self-renewal, differentiation, or quiescence are dictated by the integration of external cues from the HSC niche through cell-intrinsic signals.<sup>4</sup> Though many HSC-regulating niche factors have been identified, the role of oxygen as a critical physiological factor governing HSC biology has been a recent topic of debate.

High-resolution imaging studies have shown that the BM is a low-oxygen organ<sup>5</sup> and that quiescent HSCs display molecular characteristics associated with hypoxia (a state of insufficient oxygen availability).<sup>6</sup> However, others have shown that HSCs display a molecular signature of hypoxia regardless of their surrounding oxygen availability.<sup>7</sup> Additional studies have shown that the BM is highly vascularized and that HSCs do not

preferentially localize to low-oxygen niches<sup>5</sup> but rather reside close to blood vessels.<sup>4</sup>

Hif-1 $\alpha$  is a transcription factor that is activated in hypoxic cells and drives transcriptional programs (eg, metabolic adaptation, angiogenesis, proliferation) that allow cells to negotiate hypoxic environments. Hif-1 $\alpha$  is activated in mouse HSCs<sup>2,7</sup> and genetic ablation of *Hif-1 $\alpha$*  diminishes the hematopoietic reconstitution abilities of mouse HSCs, suggesting that Hif-1 $\alpha$  is an intrinsic regulator of HSC behavior.<sup>2</sup> Conversely, deletion of another hypoxia-inducible transcription factor, *Hif-2 $\alpha$* , either alone or in combination with *Hif-1 $\alpha$* , does not alter the ability of HSCs to reconstitute hematopoiesis.<sup>8</sup>

Using the same genetically engineered strain of *Hif-1 $\alpha$*  floxed mice used in the Takubo et al<sup>2</sup> studies, Vukovic et al have found that deletion of *Hif-1 $\alpha$*  in the hematopoietic system does not affect HSC survival or the ability of HSCs to reconstitute long-term, multilineage hematopoiesis. Using serial transplantation