Comment on Lee et al, page 2117

EFL1 deficiency: a little is better than nothing

Patrick Revy[1] and Jean Donadieu[2] | [1]Imagine Institute for Genetic Diseases; [2]Hôpital Trousseau

In this issue of *Blood*, Lee et al[1] identify 3 unrelated individuals with Shwachman-Diamond syndrome (SDS) with compound, pathogenic, heterozygous, elongation-factor–like GTPase-1 (*EFL1*) variants.

First, their findings reward their doggedness in seeking a clinically suspected genetic etiology. They observed 3 suspected SDS cases. Initially described in the 1960s, SDS typically presents with chronic neutropenia, exocrine pancreatic insufficiency, bone lesion(s), and/or developmental delay, all associated with an incredibly high rate, ∼30%, of secondary leukemic transformation.[2] The identification, ∼ 20 years ago, of deleterious biallelic *SBDS* variants as the cause of most cases of SDSs[3] stimulated relentless investigation into the function of SBDS and its role in disease. This work demonstrated that SBDS and GTPase EFL1 participated together in evicting antiassociation factor eIF6 from the nascent large 60S ribosomal subunit, enabling its fusion with 40S, which yielded the mature 80S ribosomes responsible for translation. Hence, SDS was classified as a ribosomopathy, with defective mature ribosome production.[4] Recent studies[5,6] have shown that deleterious, biallelic germline *EFL1* variants represented a previously undescribed SDS etiology and demonstrate the combined role of SBDS and EFL1 in the last step of ribosome maturation.[5,6]

Here, the authors first identified a single germline, pathogenic *EFL1* variant, in 3 SDS patients (https://www.biorxiv.org/content/10.1101/483362v1.full.pdf), but as EFL1 deficiency was previously reported to have autosomal-recessive inheritance,[5,6] finding the second pathogenic event was crucial to understanding the clinical features observed. After further painstakingly meticulous genetic investigations, the authors identified the second germline pathogenic *EFL1* variant, showing all 3 patient with SDS carried compound, heterozygous, germline, pathogenic *EFL1* variants.

The difficulty the authors encountered in detecting one of the *EFL1* variants was due to the existence of several genomic sequences closely homologous to the *EFL1* gene, including 2 *EFL1* pseudogenes (*EFL1P1* and *EFL1P2*) and segmental duplications.[6] *SBDS*, which also possesses a pseudogene (*SBDSP1*) with high sequence homology, has a strikingly similar detection difficulty.[3]

The features mentioned above make genetic diagnosis of SDS challenging. In the present study, another genetic event complicated the analysis and interpretation of the molecular etiology of SDS. Indeed, Lee et al observed disequilibrium of *EFL1*-variant frequencies in the 3 patients' blood cells. Although each variant's expected variant-allele frequency (VAF) in the context of compound heterozygosity is ∼ 50%, the *EFL1* variant's VAF was markedly underrepresented in the patients' blood cells (8.3%, 14.8%, and 36.8%), whereas the VAF of the pathogenic alleles in the patients' parents with available data was ∼50%, as expected in the context of heterozygosity.

The authors demonstrated that the disequilibrium of *EFL1*-variant allele frequencies in patients' hematopoietic cells resulted from a somatic uniparental disomy (UPD) leading to neutral copy number–loss of heterozygosity (CN-LOH) of all or part of chromosome 15 harboring the *EFL1* locus. Functional analysis of *EFL1*-knockout cell lines reconstituted with the mutated EFL1 forms, and studies in animal models (zebrafish and mice) indicated that one of the *EFL1* variants identified in the patients was functionally more deleterious than the other.

Remarkably, the somatic UPD led to replacement of the *EFL1* allele carrying the more damaging variant by the less deleterious one, rendering the cells with the somatic UPD homozygous for the less detrimental *EFL1* variant. That trait gives them a selective advantage over nonmodified cells and promotes their clonal expansion. When sought in several organs, UPD detection was limited to the blood compartment, suggesting that the somatic CN-LOH occurred only in the patients' hematopoietic system (see figure).

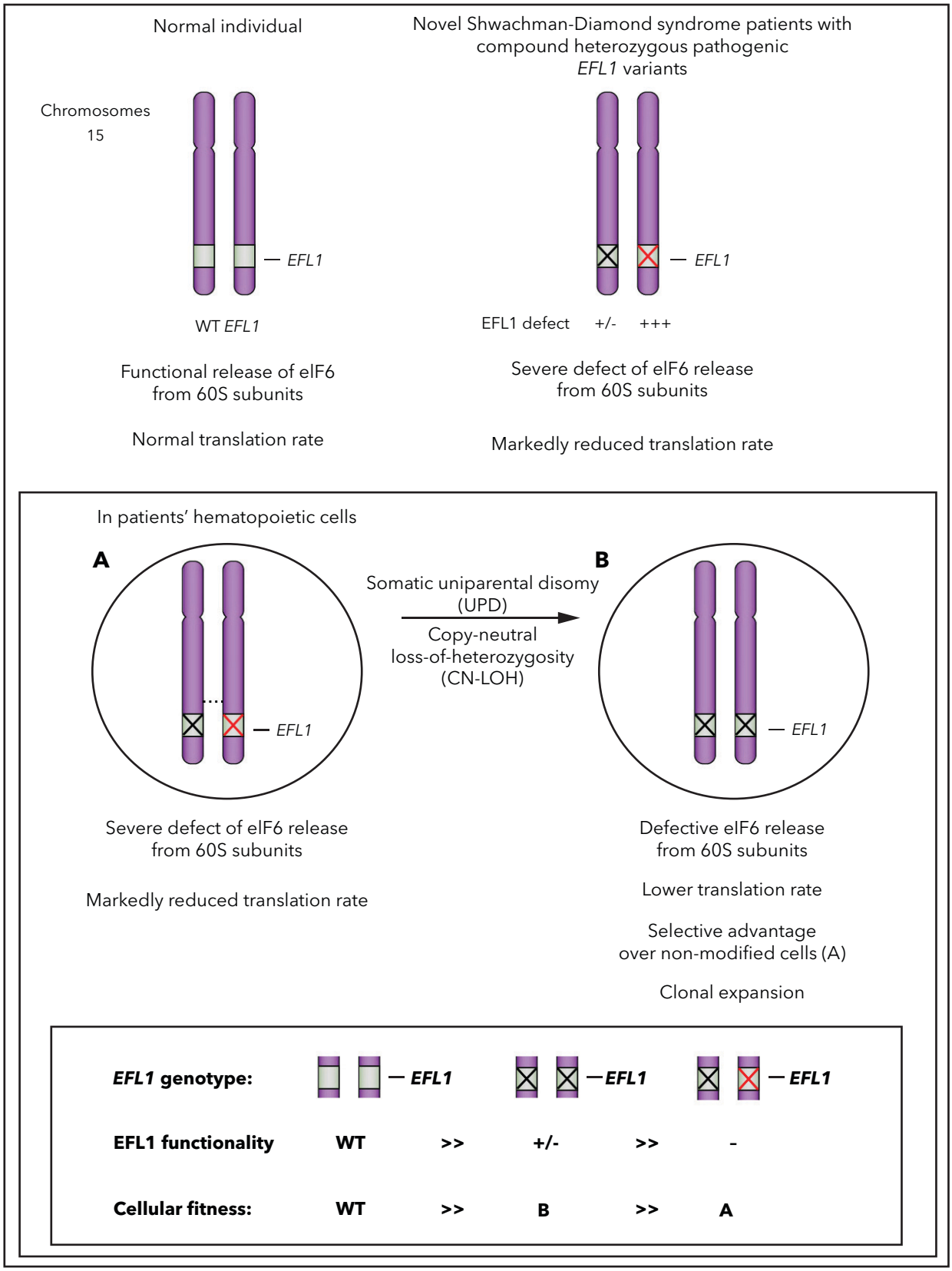
Second, beyond demonstrating the role of segmental UPD of *EFL1* in SDS, this work opens new perspectives. Somatic genetic rescue (SGR; ie, somatic genetic modifications that fully or partially counteract the deleterious effects of germline mutations, thereby providing a selective advantage to the unmodified cells) has been detected in other Mendelian hematopoietic diseases. In these rare cases, SGR has been shown to substantially blunt the patients' clinical features and even completely cure the disease.[7]

In this study, the UPD CN-LOH can be considered a partial SGR, resulting in blood compartment mosaicism. Although the frequencies of UPD CN-LOH–positive blood cells were relatively high (85% and 80%), no clinical benefit was evident at the time of evaluation.

Parikh and colleagues[8] reported UPD CN-LOH in chromosome 7, around the *SBDS* gene in hematopoietic cells from a patient with SDS carrying a compound, heterozygous, germline, pathogenic *SBDS* variant. Their finding is reminiscent of the current report of a CN-LOH replacement of a more damaging allele that acted as a null allele by the hypomorphic *SBDS* variant.

More recently, somatic genetic events in *EIF6* (including interstitial chromosomal deletion, reciprocal translocation, and point mutations), that either sharply lowered eIF6 production or affected its function, have been shown to represent another type of SGR in the hematopoietic cells from patients with SDS (Tan S, Kermasson L, Hilcenko C, et al, manuscript submitted 2021).[9]

The findings of Lee et al further support the notion that several distinct somatic genetic events can modify the fate of the



Segmental UPD of *EFL1* in SDS: a schematic representation. Normal individual has 2 copies of wild-type (WT) *EFL1* alleles (upper left). In the pathological situation (upper right), both *EFL1* alleles carry deleterious/pathogenic variants (the X black allele is mildly defective, whereas the X red one is profoundly defective). Uniparental disomy (lower scheme) leads to the substitution of the most deleterious *EFL1* allele (red X allele) by the less deleterious one (black X allele), contributing to mitigate the functional consequences of the most severe allele.

blood cells of patients with SDS, and likely impact their clinical evolution.

In the future, SGR will probably be detected in other conditions. Thus, one can imagine that cheaper and improved sensitive sequencing methods will enable the systematic search for or longitudinal follow-up of SGR events. This would represent considerable progress of patient-centered medicine by helping adopt the best therapeutic decisions for patients with Mendelian hematopoietic diseases, including SDS.

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

REFERENCES

1. Lee S, Shin CH, Lee J, et al. Somatic uniparental disomy mitigates the most damaging *EFL1* allele combination in Shwachman-Diamond syndrome. *Blood*. 2021;138(21):2117-2128.
2. Donadieu J, Fenneteau O, Beaupain B, et al; Associated investigators of the French Severe Chronic Neutropenia Registry. Classification of and risk factors for hematologic complications in a French national cohort of 102 patients with Shwachman-Diamond syndrome. *Haematologica*. 2012;97(9):1312-1319.
3. Boocock GR, Morrison JA, Popovic M, et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. *Nat Genet*. 2003;33(1):97-101.
4. Warren AJ. Molecular basis of the human ribosomopathy Shwachman-Diamond syndrome. *Adv Biol Regul*. 2018;67:109-127.
5. Stepensky P, Chacón-Flores M, Kim KH, et al. Mutations in *EFL1*, an *SBDS* partner, are associated with infantile pancytopenia, exocrine pancreatic insufficiency and skeletal anomalies in aShwachman-Diamond like syndrome. *J Med Genet*. 2017;54(8):558-566.
6. Tan S, Kermasson L, Hoslin A, et al. EFL1 mutations impair eIF6 release to cause Shwachman-Diamond syndrome. *Blood*. 2019;134(3):277-290.
7. Revy P, Kannengiesser C, Fischer A. Somatic genetic rescue in Mendelian haematopoietic diseases. *Nat Rev Genet*. 2019;20(10):582-598.
8. Parikh S, Perdigones N, Paessler M, et al. Acquired copy number neutral loss of heterozygosity of chromosome 7 associated with clonal haematopoiesis in a patient with Shwachman-Diamond syndrome. *Br J Haematol*. 2012;159(4):480-482.
9. Tan S, Kermasson L, Hilcenko C, et al. Somatic genetic rescue of a germline ribosome assembly defect. *Nat Commun*. 2021;12(1):1334-1351.

DOI 10.1182/blood.2021012724

THROMBOSIS AND HEMOSTASIS

Comment on Skjeflo et al, page 2129

Complementing venous thromboembolism, a risky move

Kristen M. Sanfilippo | Washington University School of Medicine in St Louis; St Louis Veterans Administration Medical Center

In this issue of *Blood*, Skjeflo et al demonstrate an association between plasma complement factor 5 (C5) levels and risk of future venous thromboembolism (VTE) in a nested case-control study of patients enrolled in the Tromsø population-based cohort study.[1]

Resembling the coagulation cascade in organization, the complement system is a key component of innate immunity. Experimental studies have demonstrated complex interplay between the complement system and the coagulation cascade promoting VTE. As with the coagulation cascade, there are 3 pathways, which can activate independently or converge to cleave C5. Cleavage of C5 results in release of a potent anaphylatoxin, C5a, as well as C5b to initiate formation of C5b-C9, the membrane attack complex (MAC). Cleavage of C5 potentiates hemostasis via the actions of both C5a and the MAC. Neutrophil recruitment and priming, through increased expression of tissue factor on the neutrophils, occurs in response to C5a.[2,3] This recruitment and priming can lead to release of neutrophil extracellular traps, which further promote thrombosis through platelet aggregation and activation as well as thrombin formation.[4,5] The MAC contributes to the activation of the coagulation cascade through platelet activation leading to the release of platelet factor V and assembly of the prothrombinase complex.[6]

In a prior study, complement factor 3 (C3) levels were associated with an increased risk of future VTE.[7] As with the study by Skjeflo et al, the association of C3 with VTE remained with only slight modification of the risk estimates after adjustment for general inflammation, as measured by C-reactive protein, and after adjustment for body mass index. Cleavage of C3 to C3b generates C5 convertase, resulting in cleavage of C5 to C5a and C5b. Thus, higher levels of C3 result in higher levels of C5a and the MAC. In the current study, the association between C5 and risk of VTE remained, with only slight modification of the risk estimate, after similarly adjusting for C-reactive protein and body mass index. Accordingly, the agreement in findings between these 2 studies support the noted associations between complement and the development of VTE.

An additional finding of importance of this study relates to determination of drivers of plasma C5 levels. Although the acute phase reactant C-reactive protein can activate complement, the magnitude of increase in complement proteins (ie, C3) during inflammatory stimuli (eg, infection) is less pronounced. In fact, plasma C5 levels did not differ from baseline in 1 preclinical study, but were elevated at the site of inflammation.[8] In this study, the authors found only a weak linear relationship between C-reactive protein and C5 levels, indicating that chronic inflammation explains only a minor part of the association between C5 and VTE. In addition, using a protein quantitative trait loci analysis, the authors found no significant SNP in either whole genome or *cis*-restricted analyses of 1 033 970 variants in the whole-exome data set. Although experimental studies suggest a role for C5 in hemostasis, Skjeflo et al demonstrate C5 as a risk factor for VTE within the general population. This transition from bench to bedside not