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To the editor:

Compound heterozygosity for 2 novel *TMEM16F* mutations in a patient with Scott syndrome

Loss of transmembrane lipid asymmetry in apoptotic cells or in activated platelets is thought to be catalyzed by a specific membrane protein named phospholipid scramblase.[1] Recently, the transmembrane protein TMEM16F was shown to be required for Ca^{2+}-induced lipid scrambling and exposure of phosphatidylserine at the cell surface.[2] The *TMEM16F* gene is located on chromosome 12 (12q12) and comprises 20 exons encoding a 910–amino acid protein with 8 transmembrane segments.[2,3] A patient with Scott syndrome, an inherited bleeding disorder caused by defective scramblase activity, was reported to be homozygous for a *TMEM16F* mutation (IVS12–1G→T) causing exon 13 skipping, frame shift, and premature termination of translation.[2]

After obtaining informed consent, we investigated another patient with Scott syndrome, a 64-year-old Welsh female with a moderate bleeding tendency. The scramblase defect in her platelets, erythrocytes, and B lymphocytes has been previously characterized.[4] The patient's genomic DNA was isolated from peripheral blood leukocytes and all *TMEM16F* exons (including splicing junctions) were amplified and sequenced. Two different mutations were identified (Figure 1A): a transition at the first nucleotide of intron 6 (IVS6 + 1G→A), disrupting the donor splice site consensus sequence of intron 6, and a single-nucleotide insertion in exon 11 (c.1219insT, cDNA numbering from the ATG), predicting a frame shift and premature termination of translation at codon 411 (Figure 1C).

To clarify the impact of the IVS6 + 1G→A mutation on *TMEM16F* pre-mRNA splicing, total RNA was isolated from the patient's blood cells using TRIzol Reagent (Invitrogen) and reverse-transcribed with the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). A *TMEM16F* cDNA amplicon spanning exons 5-8 was amplified and analyzed by agarose gel electrophoresis (Figure 1B). Whereas the normal control showed only the expected 459-bp fragment, the patient showed both the normal fragment (likely the product of the c.1219insT allele) and a shorter (345-bp) fragment. The low intensity of the patient's 459-bp band suggests that the mRNA transcribed from the c.1219insT allele is partially degraded in vivo. Sequencing of the shorter fragment showed that it lacked exon 6 (Figure 1B). Exon 6 skipping, which is attributable to the IVS6 + 1G→A mutation, predicts the in-frame deletion of 38 amino acids (residues 212-249)

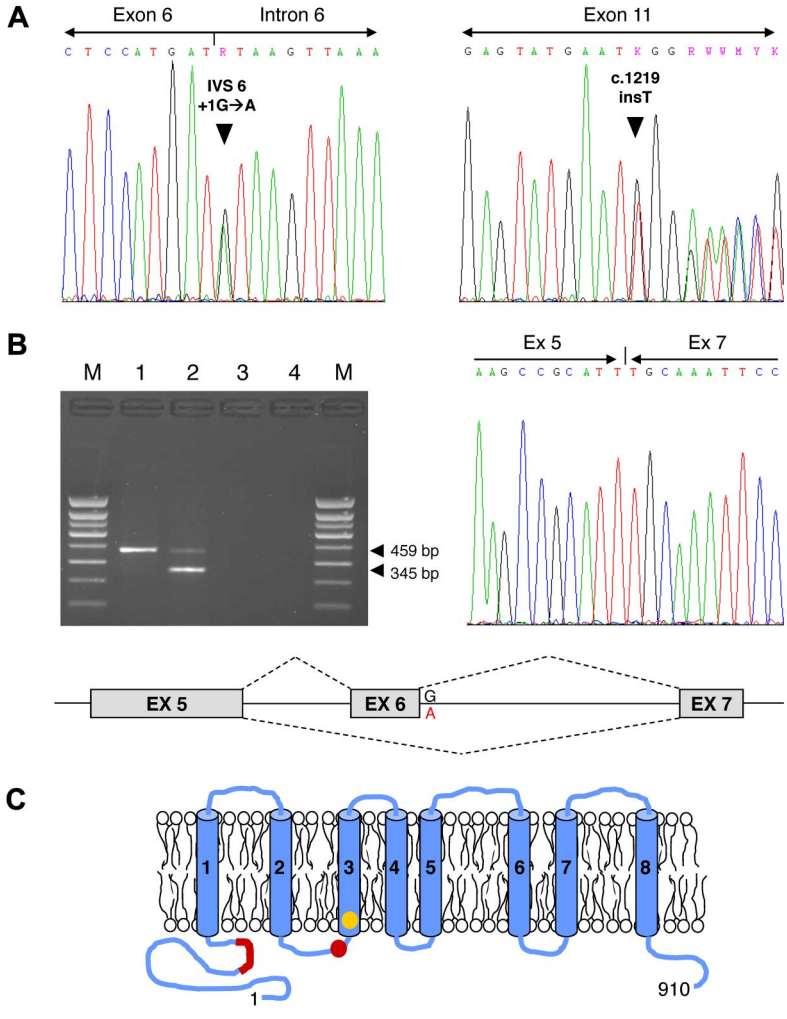

Figure 1. TMEM16F mutations. (A) *TMEM16F* mutation screening in the Scott patient under study. Identification of the *TMEM16F* IVS6 + 1G→A (left) and c.1219insT mutations (right) in the patient's genomic DNA. (B) *TMEM16F* cDNA analysis. (Top left) Amplification of a *TMEM16F* cDNA amplicon spanning exons 5-8 yielded only the expected 459-bp fragment in the normal control (lane 1), but also a shorter 345-bp fragment in the patient (lane 2). Lane 3, reverse transcription blank; lane 4, PCR blank; M, molecular weight marker. (Top right) Sequencing of the 345-bp fragment showed that it corresponds to *TMEM16F* mRNA molecules lacking exon 6 (because of the IVS6 + 1G→A mutation). (Bottom) Schematic representation of the splicing aberration (exon 6 skipping) in the patient's *TMEM16F* pre-mRNA. (C) Schematic representation of the TMEM16F protein. The positions of the previously described *TMEM16F* mutation[2] (IVS12–1G→T, predicting the truncation of the protein in the third transmembrane domain, shown in yellow) and of the mutations identified in this study (IVS6 + 1G→A, predicting the in-frame deletion of 38 amino acids in the N-terminal cytoplasmic tail, and c.1219insT, predicting the truncation of the protein between the second and third transmembrane domains, both shown in red) are indicated.

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from the N-terminal cytoplasmic domain of the protein (Figure 1C). The normal intensity of this band suggests that the stability of the mRNA transcribed from this allele is normal.

To definitely exclude the possibility that the identified mutations are polymorphisms present in the general population, 100 unrelated white individuals were genotyped for all 3 mutations by restriction analysis with BstY I (IVS12–1G→T), Rsa I (IVS6 + 1G→A, restriction site introduced with a mutagenic reverse primer) and Tsp509 I (c.1219insT), respectively (New England Biolabs). No instance of any of these mutations was found.

In conclusion, we have identified 2 novel mutations in the *TMEM16F* gene in a patient with Scott syndrome. This finding confirms the recent report[2] that the product of this gene is required for Ca^{2+}-dependent phospholipid scrambling and that loss-of-function mutations in this gene can give rise to Scott syndrome.

Elisabetta Castoldi
Department of Biochemistry, Maastricht University, Maastricht, The Netherlands

Peter W. Collins
Arthur Bloom Haemophilia Centre, School of Medicine Cardiff University, University Hospital of Wales, Cardiff, United Kingdom

Patrick L. Williamson
Department of Biology, Amherst College, Amherst, MA

Edouard M. Bevers
Department of Biochemistry, Maastricht University, Maastricht, The Netherlands

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Correspondence: Dr Edouard Bevers, Department of Biochemistry, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands; e-mail: em.bevers@maastrichtuniversity.nl.

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To the editor:

Chimerism levels after stem cell transplantation are primarily determined by the ratio of donor to host stem cells

Ever since the concept of the hematopoietic stem cell (HSC) niche was first proposed in 1978, there has been debate whether toxic ablative conditioning is required before transplantation for creating HSC niche space, or whether engraftment is merely determined by stem cell competition between donor and host stem cells. Two recent studies describe low chimerism levels after transplanting high doses of purified HSCs in unconditioned hosts[1,2] and attribute this poor engraftment to limited HSC niche spaces. This shows that in more than 30 years of research, the issue of HSC niche availability as a prerequisite for substantial chimerism has not been settled.

We directly addressed this issue in 2 different dose-response experimental models. In a congenic parent-into-F1 model, we transplanted 10×10^6, 30×10^6, and 100×10^6 unfractionated C57BL/6 CD45.1 bone marrow cells (BMCs) into unconditioned C57BL/6 CD45.1 × CD45.2 F1 recipient mice. Peripheral blood granulocytes ($Gr\text{-}1^+$) 16 weeks after transplantation demonstrated dose-dependent chimerism levels of 4.3% (± 1.7%), 8.9% (± 2.5%), and 23.2% (± 8.5%), respectively. Given that the bone marrow compartment of adult mice contains approximately 300×10^6 BMCs,[3] these results are remarkably concordant with the theoretical maximum chimerism of 3%, 9%, and 25%, respectively. In the other type of model, we transplanted 10×10^6, 30×10^6, and 100×10^6 unfractionated CD45.2 BMCs into anti-CD40-Ligand treated CD45.1 recipient mice and determined chimerism levels 20 weeks after transplantation in the peripheral blood $Gr\text{-}1^+$ population and in the $c\text{-}Kit^{hi}/Lin^-/Sca\text{-}1^+$ (KLS) population in the bone marrow, which is highly enriched for HSCs. Anti–CD40-ligand treatment served to prevent rejection resulting from CD45 polymorphism. $Gr\text{-}1^+$ chimerism levels showed a similar linear relation to the number of BMCs transplanted as in the parent-into-F1 model. Moreover, a linear relation was also observed in the bone marrow KLS fraction (Figure 1A). These data show that even after transplantation of very high doses BMCs (100×10^6), the majority of donor HSC indeed engraft. In a subsequent experiment, we assessed donor chimerism in the more primitive $KLS/CD34^-$ BMC population, containing the HSC fraction. Twenty weeks after transplanting 100×10^6 unfractionated CD45.2 BMCs into unconditioned CD45.1 mice, we detected similar high levels of donor chimerism within the $Gr\text{-}1^+$, the KLS BMC population, and the $KLS/CD34^-$ BMC population (Figure 1B).

We previously reported similar findings after transplanting high doses of BMCs in a hybrid resistance model[4] and in a major mismatched setting after immunologic conditioning,[5] indicating that our findings are not model-dependent.

Our data show that high levels of engraftment can be obtained without myeloablation and importantly show that the availability of niche space is not a limiting factor in HSC engraftment. The linear relationship between engraftment and donor to host stem cells ratios may be lost on the use of enriched or purified populations of stem cells, underscoring the important role of accessory cells. This may explain why this issue remained unresolved for such a long time. We conclude that strategies aimed at enhancing donor

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