HHS Public Access

Author manuscript

Arterioscler Thromb Vasc Biol. Author manuscript; available in PMC 2018 February 01.

Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2017 February ; 37(2): 264–270. doi:10.1161/ATVBAHA.116.308614.

Novel Thrombotic Function of a Human SNP in *STXBP5* Revealed by CRISPR-Cas9 Gene Editing in Mice

Qiuyu Martin Zhu[1], **Kyung Ae Ko**[1], **Sara Ture**[1], **Michael A. Mastrangelo**[1], **Ming-Huei Chen**[2], **Andrew D. Johnson**[2], **Christopher J. O'Donnell**[2,3], **Craig N. Morrell**[1], **Joseph M. Miano**[1], and **Charles J. Lowenstein**[1]

[1]Aab Cardiovascular Research Institute, Department of Medicine, University of Rochester Medical Center, Rochester, NY 14642, USA

[2]Division of Intramural Research, National Heart, Lung and Blood Institute (NHLBI), National Institutes of Health, and NHLBI's Framingham Heart Study, Framingham, MA 01702, USA

[3]Cardiovascular Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

Abstract

Objective—To identify and characterize the effect of a single nucleotide polymorphism (SNP) in the *STXBP5* locus that is associated with altered thrombosis in humans. Genome wide association studies (GWAS) have identified numerous SNPs associated with human thrombotic phenotypes, but determining the functional significance of an individual candidate SNP can be challenging, particularly when in vivo modeling is required. Recent GWAS led to the discovery of STXBP5 as a regulator of platelet secretion in humans. Further clinical studies have identified genetic variants of *STXBP5* that are linked to altered plasma von Willebrand factor (VWF) levels and thrombosis in humans, but the functional significance of these variants in *STXBP5* is not understood.

Approach and Results—We used CRISPR-Cas9 techniques to produce a precise mouse model carrying a human coding SNP *rs1039084* (encoding human *p. N436S*) in the *STXBP5* locus associated with decreased thrombosis. Mice carrying the orthologous human mutation (encoding *p. N437S* in mouse STXBP5) have lower plasma VWF levels, decreased thrombosis, and decreased platelet secretion compared to wild-type mice. This thrombosis phenotype recapitulates the phenotype of humans carrying the minor allele of *rs1039084*. Decreased plasma VWF and platelet activation may partially explain the decreased thrombotic phenotype in mutant mice.

Conclusions—Using precise mammalian genome editing, we have identified a human non-synonymous SNP *rs1039084* in the *STXBP5* locus as a causal variant for a decreased thrombotic phenotype. CRISPR-Cas9 genetic editing facilitates the rapid and efficient generation of animals to study the function of human genetic variation in vascular diseases.

Correspondence to Charles J. Lowenstein, Box CVRI, 601 Elmwood Avenue, Rochester, NY 14642, USA. Tel: (585) 276-7723. Fax: (585) 276-9830. charles_lowenstein@urmc.rochester.edu.

Disclosures

None.

Keywords

genome-wide association studies (GWAS); thrombosis; platelets; von Willebrand factor; CRISPR-Cas9

Subject codes

Vascular Biology; Vascular Disease; Platelets; Genetically Altered and Transgenic Models; Genetics

Introduction

Vascular injury and thrombosis play a central role in cardiovascular diseases such as myocardial infarction, stroke, and venous thromboembolism. Platelets respond to vascular injury by exocytosis, releasing pro-thrombotic factors such as von Willebrand factor (VWF) that mediates platelet adherence to the vessel wall. Plasma VWF levels are associated with thrombosis and the risk of cardiovascular events.[1] An enhanced understanding of exocytosis may lead to novel treatments for thrombotic diseases.

Recent genome wide association studies (GWAS) by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium and others identified novel genetic loci associated with altered plasma VWF levels in humans.[2–6] These loci may contain novel genes whose products regulate endothelial or platelet exocytosis.[7] For example, the CHARGE Consortium identified over 50 single nucleotide polymorphisms (SNPs) linked to altered VWF levels that are located within or near the gene encoding syntaxin-binding protein 5 (*STXBP5*).[2–6] This association of genetic variants in *STXBP5* with VWF levels and with thrombosis risk was strengthened by additional human studies.[4, 8, 9] More recently, we and others found STXBP5 is a novel regulator of platelet granule exocytosis and thrombosis.[10–12]

Multiple genetic variants associated with altered VWF levels lie within the *STXBP5* locus.[2–5] However, the role of these specific genetic variants in determining VWF levels, platelet function and thrombosis is unknown. The traditional approach for functional study of genetic variants in vivo is to generate mouse lines carrying these mutations by homologous recombination in mouse embryonic stem cells, a process which can take up to one year or more with some targeting never achieved. However, the genetic techniques using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) system permit the rapid generation of novel animal models.[13–15] Precise genome editing can be achieved by the CRISPR-Cas9 system, which is comprised of a *Cas9* endonuclease mRNA, a single-guide RNA (sgRNA) that directs Cas9-mediated DNA double strand break (DSB) at the genomic region of interest, and an exogenous single-strand donor oligonucleotide (ssODN) on which desired mutation can be synthesized and integrated into DSB site by homology directed repair (HDR).[16–18] The unprecedented ease and efficiency of the CRISPR-Cas9 system has permitted us to investigate the effect of a specific *STXBP5* genetic variant upon exocytosis and thrombosis in vivo.

Materials and Methods

Detailed information is available in the online-only Data Supplement.

Results

We first determined the individual SNPs identified by the CHARGE Consortium that are most likely to affect STXBP5 function. The *STXBP5* SNP associated with altered VWF levels with the highest genome-wide significance ($P = 1.16 \times 10^{-22}$) is *rs9390459* (hg19 chr6: g.147680359 G>A), a synonymous variant in *STXBP5*.[2] Although synonymous, it is conceivable that *rs9390459* could affect STXBP5 function via epigenetic mechanisms. Alternatively, the SNP may serve as a proxy that links to other causal, non-synonymous variants responsible for changes in VWF levels via post-transcriptional mechanism.[19] Therefore we searched for possible explanations for how the synonymous variant *rs9390459* might affect VWF levels and thrombosis: potential epigenetic effects, potential enhancer effects, gene dosage effect via expression quantitative trait loci (eQTLs), or potential linkage disequilibrium with non-synonymous variants.

First we searched for a potential link between *rs9390459* and epigenetic features by examining *rs9390459* in the UCSC genome browser, seeking potential chromatin modifications including methylation and acetylation, or transcription factor binding sites. Among all 7 cell types (GM12878, H1-hESC, HSMM, HUVEC, K562, NHEK, and NHLF) of the Encyclopedia of DNA Elements (ENCODE) Project,[20] the genomic DNA containing SNP *rs9390459* is uniformly devoid of histone markers that are associated with gene expression or transcriptional regulation. Furthermore, among 161 transcription factors assayed by ENCODE, no transcription factor binding signals were identified in the cell type (HUVEC) relevant to plasma VWF (Supplementary Figure I). The annotations of the noncoding genome at *rs9390459* is also unremarkable for endothelial cells in HaploReg (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php)[21]. We also did not detect microRNA, lncRNA, or predicted genes that overlap with or near *rs9390459* besides *STXBP5* (data not shown).

Next we investigated possible interactions of *rs9390459* with distal DNA, since non-coding DNA variants can interact with distant enhancers, leading to human disease.[22] We investigated potential DNA 3-dimensional interactions of *rs9390459* using genome-wide in situ Hi-C data mapped on HUVEC with kilobase-resolution.[23] Within a ~7 million bp genomic DNA window flanking *rs9390459*, putative DNA looping structures can be recognized with experimentally-identified DNA contact domains. However, *rs9390459* is not associated with DNA loop anchors, contact domains, or distinct patterns of histone markers linked to promoters or enhancers (Supplementary Figure II). While Hi-C technique captures interactions genome-wide[24], it does not provide functional information of the contacting sites and undermined by low resolution. Therefore, we searched for datasets of chromatin interaction analysis with paired-end tag sequencing (ChIA-PET) that focus on parts of the genome that have chromatin-binding proteins and functional implications in high resolution[25]. Using the well annotated ChIA-PET datasets on human K562 cells, distal chromatin interactions were found associated with insulator protein (CTCF), the

transcription machinery (RNA-Pol II), and chromosome repair and cohesion machinery (RAD21) outside of *rs9390459* (Supplementary Figure III). The locus of *rs9390459* is found in a region that lacks distal chromatin interaction associated with these proteins. Together with HUVEC Hi-C data, the evidence does not support that *rs9390459* is likely to affect the *STXBP5* locus via distal chromatin interactions.

We then searched for eQTLs using the Genotype-Tissue Expression (GTEx) project data (http://www.gtexportal.org/home/) containing 8555 genome-wide gene expression profiles in 53 human tissues.[26] SNP *rs9390459* is not significantly correlated with any gene expression trait in any profiled tissue. Taken together, our data do not support *rs9390459* as a non-coding variant that affect DNA epigenetic modification, transcriptional factor binding, distal interaction, or expression quantitative traits.

Finally, we searched for non-synonymous variants that are in high linkage disequilibrium (LD) with the leading SNP *rs9390459*. Using data from 1000 Genomes Project (Phase 3 V5, European ancestry), we identified 56 SNPs that are in linkage disequilibrium with *rs9390459* (R^2>0.8).[27] These SNPs lie within a 193 kb genomic segment overlapping *STXBP5* (Supplementary Figure IV). Only 1 of these 56 SNP is a non-synonymous SNP, *rs1039084* (hg19 chr6: g.147635413 A>G). This non-synonymous SNP *rs1039084* is in high LD (R^2=0.92) with *rs9390459* (Supplementary Table I). The non-synonymous SNP *rs1039084* is strongly associated with plasma VWF levels in the CHARGE Consortium data ($P = 6.90 \times 10^{-22}$; Supplementary Figure V).

The non-synonymous SNP *rs1039084* encodes STXBP5 asparagine to serine substitution at position 436 in the WD40 repeat domain of STXBP5, a domain that regulates exocytosis in various cell types.[28–35] We designate this mutation as: STXBP5(N436S).

Human studies show that the non-synonymous SNP *rs1039084* minor allele G is associated with decreased VWF levels, decreased venous thrombosis risk, and increased bleeding.[4, 8, 9] These genetic and clinical studies suggest that this specific STXBP5 variant may affect STXBP5 function.

In order to identify the functional significance of the human mutation STXBP5(N436S), we previously knocked-down the endogenous STXBP5 expression in cultured human endothelial cells and found overexpression of STXBP5(N436S) decreased VWF exocytosis than wild-type STXBP5.[10] To further explore the physiological relevance of the STXBP5(N436S) mutation in vivo, we created a mouse model of this genetic variant. Mouse STXBP5 is 98% identical to human STXBP5, and mouse STXBP5(N437) corresponds to human STXBP5(N436). We employed CRISPR-Cas9 techniques to change the wild-type murine allele *AAT* encoding 437Asn to human *AGC* allele encoding 437Ser, generating a mouse designated as *Stxbp5(N437S)*.[16]

We knocked the *rs1039084* minor allele of human *STXBP5* into the orthologous mouse *Stxbp5* locus by CRISPR-Cas9 genome editing.[15, 18] Cytoplasmic injection into murine zygotes of *Streptococcus pyogenes Cas9 (SpCas9)* mRNA, purified sgRNA targeting 437Asn, and an ssODN bearing the human *rs1039084* minor allele for HDR, generated 1 of 50 pups with one allele precisely edited into the human minor allele AT > GC (Figure 1A–B

and Supplementary Table II). Precise editing was confirmed by Sanger sequencing (Figure 1C) and by restriction fragment length polymorphism (RFLP) analysis (Figure 1D). The mutant allele was transmitted through the germline, as assayed by a novel multiplex polymerase chain reaction (PCR) assay we recently developed (Figure 1E).[18]

We assessed potential mutations that could be caused by CRISPR-Cas9 editing that might lie outside of the *Stxbp5* locus. We examined 24 genomic loci with up to 3–4 mismatched nucleotides with the *Stxbp5* sgRNA that could be potential targets of Cas9 cleavage. Sanger sequencing detected no off-target cleavage in the founder mouse (Supplementary Table III). We have thus created an *Stxbp5(N437S)* mouse that carries the substitution orthologous to the human *STXBP5(N436S)* variant.

We proceeded to study the effect of the human variant on VWF levels, hemostasis, and thrombosis, using 4 – 8 wk old male mice that were *Stxbp5(N437S)* and their wild-type*(WT)* littermates.

In humans the non-synonymous SNP *rs1039084* is associated with a decrease in VWF levels.[4, 8] We tested the effect of STXBP5(N437S) upon VWF levels in mice. VWF levels are lower in homozygous (*437S/S*) mutant mice than WT mice (Fig. 2A).

In humans, the non-synonymous SNP *rs1039084* is associated with an approximately 20% decreased risk of venous thrombosis.[9] Therefore we next tested the effect of STXBP5(N437S) upon hemostasis in mice. Compared with WT mice, heterozygous (*437N/S*) and homozygous (*437S/S*) mutant mice displayed prolonged bleeding time (Figure 2B).

We then explored the effect of STXBP5(N437S) upon thrombosis in large and small arteries. First we used a Doppler probe to measure the changes in blood flow in the murine carotid artery after $FeCl_3$-induced injury. Carotid flow diminished steadily after $FeCl_3$ injury in WT mice, resulting in rapid and complete vessel occlusion (Figure 2C–D). However, the time to complete carotid occlusion was longer in *Stxbp5(N437S)* carrier mice than in WT mice (Figure 2C–D). We further characterized the thrombotic phenotype in the microvasculature in a murine mesenteric thrombosis model. Although the time to form a small (50 μm diameter) thrombus was not affected by the *Stxbp5(N437S)* mutation, mice carrying this allele had severely delayed time for complete vessel occlusion in mesenteric arterioles and venules (Figure 2E–F). Taken together, these data suggest that the genetic variant *Stxbp5(N437S)* decreases thrombosis.

We then tested the effect of STXBP5(N437S) on platelets, since prior studies show STXBP5 is required for platelet secretion.[10, 11] Stimulation of murine platelets with the physiologic agonist thrombin induced dose-dependent granule secretion, as measured by platelet factor 4 (PF4) release from α-granules (Figure 3A), and ATP and serotonin released from dense granules (Figure 3B–C). Platelets from *Stxbp5(N437S)* mice displayed significantly impaired granule secretion ex vivo in response to low dose thrombin, an effect that gradually diminished with increasing thrombin doses (Figure 3A–C). Furthermore, platelet spreading is less in platelets from *Stxbp5(N437S)* mice than in platelets from WT mice (Figure 3D and Supplementary Figure VI), a statistically significant effect that is probably not biologically

significant (Figure 3D). These data suggest that STXBP5(N437S) causes a platelet secretion defect.

Taken together, our current study provides functional evidence for the regulatory role of a genetic variant linked by GWAS to altered thrombosis.[2, 4, 5, 8] Consistent with the lower plasma VWF, prolonged bleeding and decreased venous thrombosis observed in human carriers of the *rs1039084* minor allele[4, 8, 9], *Stxbp5(N437S)* mice had lower plasma VWF, prolonged bleeding and decreased thrombosis. Our data suggest that these abnormalities are due in part to decreased plasma VWF and functional defects of *Stxbp5(N437S)* platelets.

Discussion

The major finding of our study is that a human mutation in *STXBP5* linked to VWF levels and thrombosis causes a thrombotic phenotype in mice. We used CRISPR-Cas9 gene editing to create a mouse model of human genetic variation. Mice carrying the minor allele of human SNP *rs1039084* at an orthologous locus had decreased VWF exocytosis, decreased thrombosis, increased bleeding, and decreased platelet secretion. Thus, our current study provides strong functional evidence for the regulatory role of a GWAS-derived variant on human bleeding and thrombosis phenotype.[2, 4, 5, 8]

We show that the genetic variation in STXBP5(N436) decreases platelet secretion ex vivo, but the precise mechanisms are not yet known. SNP *rs1039084* encodes N436S in the second N-terminal WD40-repeat of STXBP5, and WD40 domain has been shown to participate in the inhibition of exocytosis.[28–35] A mutation within this region may interfere with STXBP5 interactions with synaptotagmin or syntaxin, or may create a novel site for post-translational modification.[31–35] Our data showed murine heterozygotes displayed changes comparable to homozygotes, supporting the observations in humans that STXBP5(N436) may act in a gain-of-function manner.[8, 9]

VWF levels are significantly lower in STXBP5(N437) mouse plasma than in WT mouse plasma (Figure 2A). This murine phenotype recapitulates the human phenotype, since *rs1039084* is associated with lower plasma VWF levels in humans.[4, 8] The effect of this SNP upon VWF levels is even greater in subjects with coronary artery disease or stroke or peripheral arterial disease than in healthy control subjects.[4] The underlying causes of this increased effect of mutations in STXBP5 upon VWF levels in subjects with arterial disease are unclear. Further studies in human subjects are needed to clarify the relationship between *rs1039084* and VWF levels and atherosclerosis.

One limitation of the study is that currently there is no effective method to identify off-target mutations in genome-edited animals[15]. Off-target surveys such as GUIDE-seq found that in cells the majority of off-target sites were not detected by computational methods, and robust experiment-based off-target detection is required to efficiently identify real off-target edits.[36] However, genome-sequencing-based evaluation showed off-target mutations are rare in Cas9-modified mice. Even if off-targets are occasionally found in the Cas9-modified founder mouse, they can be readily bred away by crossing with wild-type animals[18], or completely avoided by simply generating a new mouse line using an alternative gRNA.

There are important strategies to limit off-targets in animal genome-targeting design.[15, 37] In addition, the advantage of a genome-edited animal model is that any off-target effects can be effectively controlled by using littermate controls. The potential off-target effects from the current study are likely to be insignificant, although robust off-target detection methods such as GUIDE-seq in an in vivo setting would be highly informative to accurately assess the off-target effects of future genome-edited mouse models.

In conclusion, our study reveals the functional relevance of a candidate SNP identified by GWAS. Our data suggest that genetic variation within *STXBP5* is a genetic risk for bleeding and thrombotic diseases in humans. Our study demonstrates that CRISPR-Cas9 gene editing in mice is a rapid approach to studying the functional significance of genetic variants associated with thrombosis. This approach permits efficient, inexpensive, and rigorous disease modeling in animals, paving the way for rapidly testing the functional consequences and clinical relevance of genetic variations that are linked to human vascular diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank L. Gan and M. Deng of the University of Rochester Mouse Genome Editing Resource for their zygote microinjection and embryo transfers. We thank N. L. Smith for advice and assistance.

Sources of Funding

This work was supported by NIH/NHLBI grant HL108372 (C.J.L.), HL124042 (C.J.L.), HL117907 (J.M.M.), and HL124018 (C.N.M.); the Howard Hughes Medical Institute “Med-into-Grad” Fellowship in Cardiovascular Sciences (C.J.L., Q.Z.); the American Heart Association 14GRNT19020033 (C.J.L.) and 13PRE17050105 (Q.Z.).

Abbreviations

Cas9	CRISPR-associated protein 9
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
CRISPR	clustered regularly interspaced short palindromic repeats
DSB	double-strand break
ENCODE	Encyclopedia of DNA Elements
eQTL	expression quantitative trait locus
GTEx	Genotype-Tissue Expression
GWAS	genome wide association studies
HDR	homology-directed repair
LD	linkage disequilibrium
PF4	platelet factor 4

RFLP	restriction fragment length polymorphism
sgRNA	single-guide RNA
SNP	single-nucleotide polymorphism
ssODN	single-strand donor oligonucleotide
STXBP5	syntaxin-binding protein 5
tracrRNA	transactivating crRNA
VWF	von Willebrand factor

References

1. Spiel AO, Gilbert JC, Jilma B. Von willebrand factor in cardiovascular disease: Focus on acute coronary syndromes. Circulation. 2008; 117:1449–1459. [PubMed: 18347221]
2. Smith NL, Chen MH, Dehghan A, Strachan DP, Basu S, Soranzo N, Hayward C, Rudan I, Sabater-Lleal M, Bis JC, de Maat MP, Rumley A, Kong X, Yang Q, Williams FM, Vitart V, Campbell H, Malarstig A, Wiggins KL, Van Duijn CM, McArdle WL, Pankow JS, Johnson AD, Silveira A, McKnight B, Uitterlinden AG, Aleksic N, Meigs JB, Peters A, Koenig W, Cushman M, Kathiresan S, Rotter JI, Bovill EG, Hofman A, Boerwinkle E, Tofler GH, Peden JF, Psaty BM, Leebeek F, Folsom AR, Larson MG, Spector TD, Wright AF, Wilson JF, Hamsten A, Lumley T, Witteman JC, Tang W, O'Donnell CJ. Wellcome Trust Case Control C. Novel associations of multiple genetic loci with plasma levels of factor vii, factor viii, and von willebrand factor: The charge (cohorts for heart and aging research in genome epidemiology) consortium. Circulation. 2010; 121:1382–1392. [PubMed: 20231535]
3. Antoni G, Oudot-Mellakh T, Dimitromanolakis A, Germain M, Cohen W, Wells P, Lathrop M, Gagnon F, Morange PE, Tregouet DA. Combined analysis of three genome-wide association studies on vwf and fviii plasma levels. BMC Med Genet. 2011; 12:102. [PubMed: 21810271]
4. van Loon JE, Leebeek FW, Deckers JW, Dippel DW, Poldermans D, Strachan DP, Tang W, O'Donnell CJ, Smith NL, de Maat MP. Effect of genetic variations in syntaxin-binding protein-5 and syntaxin-2 on von willebrand factor concentration and cardiovascular risk. Circ Cardiovasc Genet. 2010; 3:507–512. [PubMed: 21156930]
5. Sanders YV, van der Bom JG, Isaacs A, Cnossen MH, de Maat MP, Laros-van Gorkom BA, Fijnvandraat K, Meijer K, van Duijn CM, Mauser-Bunschoten EP, Eikenboom J, Leebeek FW, Wi Nsg. Clec4m and stxbp5 gene variation contribute to von willebrand factor level variation in von willebrand disease. Journal of thrombosis and haemostasis : JTH. 2015
6. van Loon J, Dehghan A, Weihong T, Trompet S, McArdle WL, Asselbergs FF, Chen MH, Lopez LM, Huffman JE, Leebeek FW, Basu S, Stott DJ, Rumley A, Gansevoort RT, Davies G, Wilson JJ, Witteman JC, Cao X, de Craen AJ, Bakker SJ, Psaty BM, Starr JM, Hofman A, Wouter Jukema J, Deary IJ, Hayward C, van der Harst P, Lowe GD, Folsom AR, Strachan DP, Smith N, de Maat MP, O'Donnell C. Genome-wide association studies identify genetic loci for low von willebrand factor levels. Eur J Hum Genet. 2015
7. Rydz N, Swystun LL, Notley C, Paterson AD, Riches JJ, Sponagle K, Boonyawat B, Montgomery RR, James PD, Lillicrap D. The c-type lectin receptor clec4m binds, internalizes, and clears von willebrand factor and contributes to the variation in plasma von willebrand factor levels. Blood. 2013; 121:5228–5237. [PubMed: 23529928]
8. van Loon JE, Sanders YV, de Wee EM, Kruip MJ, de Maat MP, Leebeek FW. Effect of genetic variation in stxbp5 and stx2 on von willebrand factor and bleeding phenotype in type 1 von willebrand disease patients. PLoS One. 2012; 7:e40624. [PubMed: 22792389]
9. Smith NL, Rice KM, Bovill EG, Cushman M, Bis JC, McKnight B, Lumley T, Glazer NL, van Hylckama Vlieg A, Tang W, Dehghan A, Strachan DP, O'Donnell CJ, Rotter JI, Heckbert SR, Psaty

BM, Rosendaal FR. Genetic variation associated with plasma von willebrand factor levels and the risk of incident venous thrombosis. Blood. 2011; 117:6007–6011. [PubMed: 21163921]

10. Zhu Q, Yamakuchi M, Ture S, de la Luz Garcia-Hernandez M, Ko KA, Modjeski KL, LoMonaco MB, Johnson AD, O'Donnell CJ, Takai Y, Morrell CN, Lowenstein CJ. Syntaxin-binding protein stxbp5 inhibits endothelial exocytosis and promotes platelet secretion. J Clin Invest. 2014; 124:4503–4516. [PubMed: 25244095]
11. Ye S, Huang Y, Joshi S, Zhang J, Yang F, Zhang G, Smyth SS, Li Z, Takai Y, Whiteheart SW. Platelet secretion and hemostasis require syntaxin-binding protein stxbp5. J Clin Invest. 2014; 124:4517–4528. [PubMed: 25244094]
12. Lillicrap D. Syntaxin-binding protein 5 exocytosis regulation: Differential role in endothelial cells and platelets. J Clin Invest. 2014; 124:4231–4233. [PubMed: 25244090]
13. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: Zfns, talens, and crispr-cas9. The Journal of clinical investigation. 2014; 124:4154–4161. [PubMed: 25271723]
14. Hsu PD, Lander ES, Zhang F. Development and applications of crispr-cas9 for genome engineering. Cell. 2014; 157:1262–1278. [PubMed: 24906146]
15. Miano JM, Zhu QM, Lowenstein CJ. A crispr path to engineering new genetic mouse models for cardiovascular research. Arterioscler Thromb Vasc Biol. 2016; 36:1058–1075. [PubMed: 27102963]
16. Yang H, Wang H, Jaenisch R. Generating genetically modified mice using crispr/cas-mediated genome engineering. Nat Protoc. 2014; 9:1956–1968. [PubMed: 25058643]
17. Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, Jaenisch R. One-step generation of mice carrying mutations in multiple genes by crispr/cas-mediated genome engineering. Cell. 2013; 153:910–918. [PubMed: 23643243]
18. Han Y, Slivano OJ, Christie CK, Cheng AW, Miano JM. Crispr-cas9 genome editing of a single regulatory element nearly abolishes target gene expression in mice--brief report. Arterioscler Thromb Vasc Biol. 2015; 35:312–315. [PubMed: 25538209]
19. Sauna ZE, Kimchi-Sarfaty C. Understanding the contribution of synonymous mutations to human disease. Nat Rev Genet. 2011; 12:683–691. [PubMed: 21878961]
20. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012; 489:57–74. [PubMed: 22955616]
21. Ward LD, Kellis M. Haploreg: A resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 2012; 40:D930–934. [PubMed: 22064851]
22. Visel A, Rubin EM, Pennacchio LA. Genomic views of distant-acting enhancers. Nature. 2009; 461:199–205. [PubMed: 19741700]
23. Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, Aiden EL. A 3d map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell. 2014; 159:1665–1680. [PubMed: 25497547]
24. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, Sandstrom R, Bernstein B, Bender MA, Groudine M, Gnirke A, Stamatoyannopoulos J, Mirny LA, Lander ES, Dekker J. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science. 2009; 326:289–293. [PubMed: 19815776]
25. Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, Chew EG, Huang PY, Welboren WJ, Han Y, Ooi HS, Ariyaratne PN, Vega VB, Luo Y, Tan PY, Choy PY, Wansa KD, Zhao B, Lim KS, Leow SC, Yow JS, Joseph R, Li H, Desai KV, Thomsen JS, Lee YK, Karuturi RK, Herve T, Bourque G, Stunnenberg HG, Ruan X, Cacheux-Rataboul V, Sung WK, Liu ET, Wei CL, Cheung E, Ruan Y. An oestrogen-receptor-alpha-bound human chromatin interactome. Nature. 2009; 462:58–64. [PubMed: 19890323]
26. Consortium GT. Human genomics. The genotype-tissue expression (gtex) pilot analysis: Multitissue gene regulation in humans. Science. 2015; 348:648–660. [PubMed: 25954001]
27. Arnold M, Raffler J, Pfeufer A, Suhre K, Kastenmuller G. Snipa: An interactive, genetic variant-centered annotation browser. Bioinformatics. 2015; 31:1334–1336. [PubMed: 25431330]

28. Pobbati AV, Razeto A, Boddener M, Becker S, Fasshauer D. Structural basis for the inhibitory role of tomosyn in exocytosis. J Biol Chem. 2004; 279:47192–47200. [PubMed: 15316007]
29. Baba T, Sakisaka T, Mochida S, Takai Y. Pka-catalyzed phosphorylation of tomosyn and its implication in ca2+-dependent exocytosis of neurotransmitter. J Cell Biol. 2005; 170:1113–1125. [PubMed: 16186257]
30. Yizhar O, Lipstein N, Gladycheva SE, Matti U, Ernst SA, Rettig J, Stuenkel EL, Ashery U. Multiple functional domains are involved in tomosyn regulation of exocytosis. J Neurochem. 2007; 103:604–616. [PubMed: 17666050]
31. Sakisaka T, Yamamoto Y, Mochida S, Nakamura M, Nishikawa K, Ishizaki H, Okamoto-Tanaka M, Miyoshi J, Fujiyoshi Y, Manabe T, Takai Y. Dual inhibition of snare complex formation by tomosyn ensures controlled neurotransmitter release. J Cell Biol. 2008; 183:323–337. [PubMed: 18936251]
32. Yamamoto Y, Mochida S, Miyazaki N, Kawai K, Fujikura K, Kurooka T, Iwasaki K, Sakisaka T. Tomosyn inhibits synaptotagmin-1-mediated step of ca2+-dependent neurotransmitter release through its n-terminal wd40 repeats. J Biol Chem. 2010; 285:40943–40955. [PubMed: 20978127]
33. Williams AL, Bielopolski N, Meroz D, Lam AD, Passmore DR, Ben-Tal N, Ernst SA, Ashery U, Stuenkel EL. Structural and functional analysis of tomosyn identifies domains important in exocytotic regulation. J Biol Chem. 2011; 286:14542–14553. [PubMed: 21330375]
34. Bielopolski N, Lam AD, Bar-On D, Sauer M, Stuenkel EL, Ashery U. Differential interaction of tomosyn with syntaxin and snap25 depends on domains in the wd40 beta-propeller core and determines its inhibitory activity. J Biol Chem. 2014; 289:17087–17099. [PubMed: 24782308]
35. Yu H, Rathore SS, Gulbranson DR, Shen J. The n- and c-terminal domains of tomosyn play distinct roles in soluble n-ethylmaleimide-sensitive factor attachment protein receptor binding and fusion regulation. J Biol Chem. 2014; 289:25571–25580. [PubMed: 25063806]
36. Tsai SQ, Zheng Z, Nguyen NT, Liebers M, Topkar VV, Thapar V, Wyvekens N, Khayter C, Iafrate AJ, Le LP, Aryee MJ, Joung JK. Guide-seq enables genome-wide profiling of off-target cleavage by crispr-cas nucleases. Nat Biotechnol. 2015; 33:187–197. [PubMed: 25513782]
37. Zhang XH, Tee LY, Wang XG, Huang QS, Yang SH. Off-target effects in crispr/cas9-mediated genome engineering. Mol Ther Nucleic Acids. 2015; 4:e264. [PubMed: 26575098]

Highlights

- We used CRISPR-Cas9 genome editing to produce a novel mouse model of a human STXBP5 SNP linked to thrombosis.
- Mice carrying the minor allele of human SNP *rs1039084* encoding STXBP5(N437S) have decreased plasma levels of VWF.
- STXBP5(N437S) mice displayed defects in hemostasis, thrombosis, and platelet function.
- Our study validates the functional relevance of a candidate SNP identified by GWAS, and suggests that variation within STXBP5 is a genetic risk for venous thromboembolic disease.

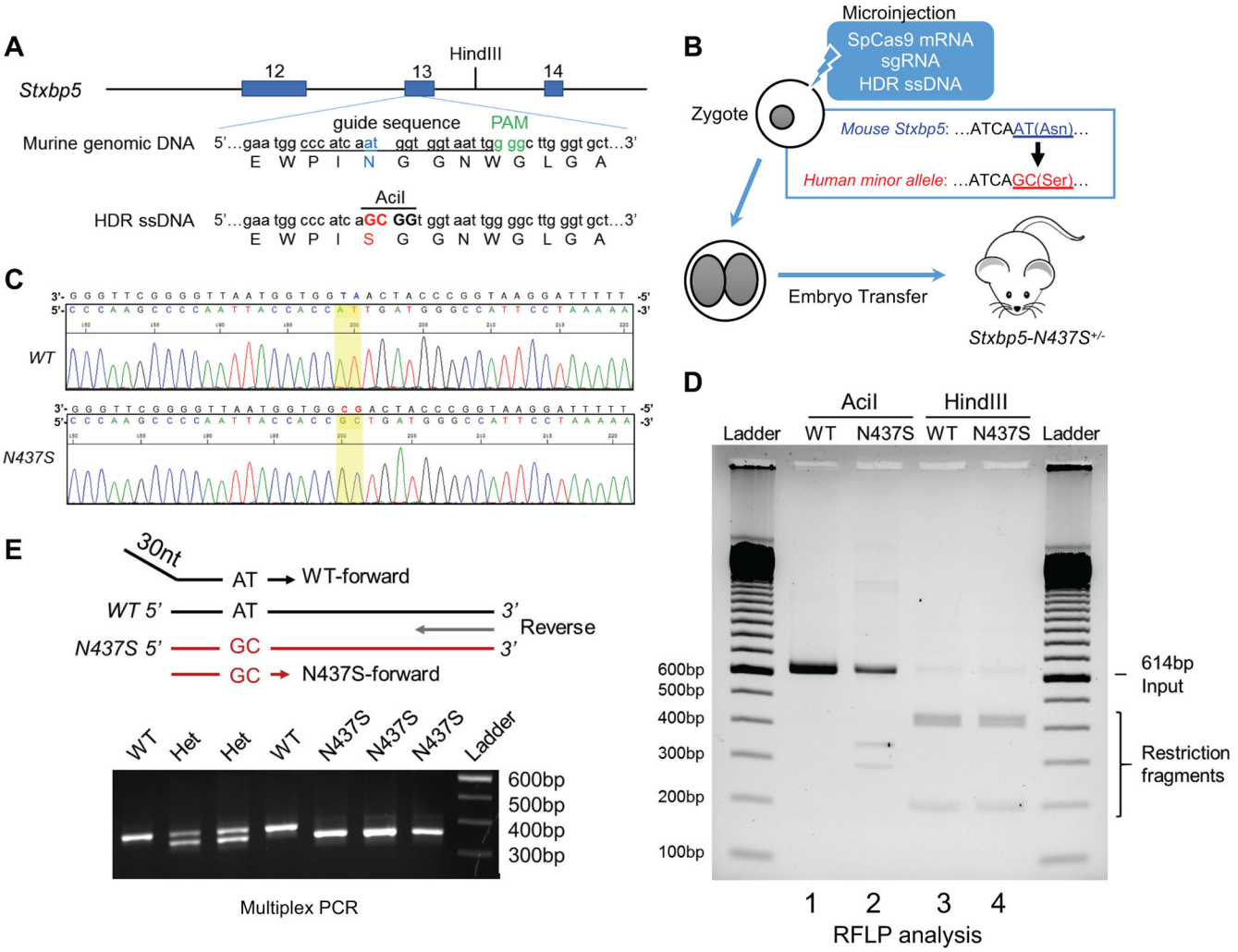

Figure 1.

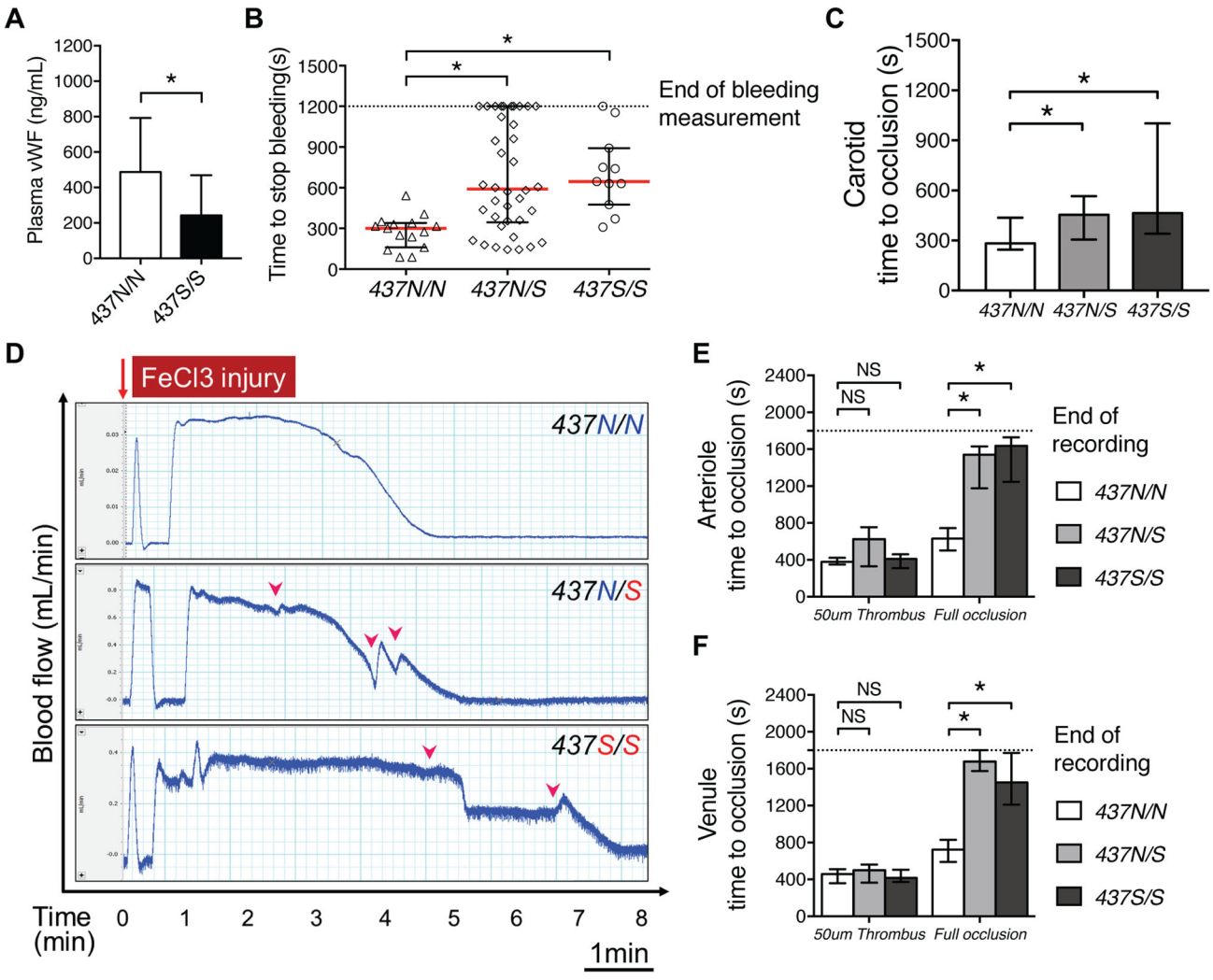

Figure 2.

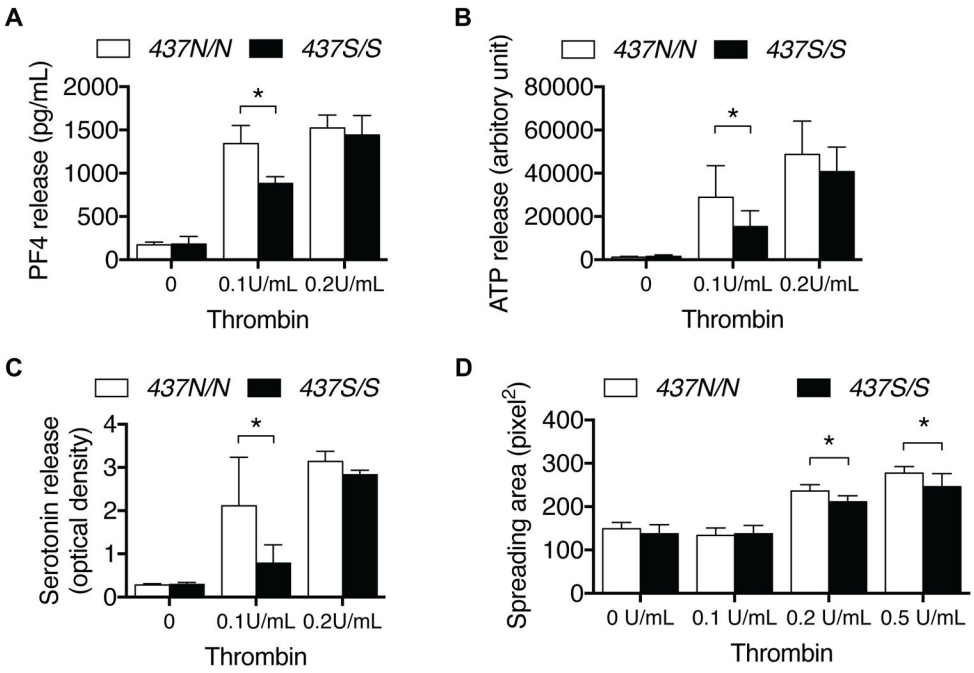

Figure 3.