Functional genomics in zebrafish permits rapid characterization of novel platelet membrane proteins

In this study, we demonstrate the suitability of the vertebrate *Danio rerio* (zebrafish) for functional screening of novel platelet genes in vivo by reverse genetics. Comparative transcript analysis of platelets and their precursor cell, the megakaryocyte, together with nucleated blood cell elements, endothelial cells, and erythroblasts, identified novel platelet membrane proteins with hitherto unknown roles in thrombus formation. We determined the phenotype induced by antisense morpholino oligonucleotide (MO)-based knockdown of 5 of these genes in a laser-induced arterial thrombosis model. To validate the model, the genes for platelet glycoprotein (GP) IIb and the coagulation protein factor VIII were targeted. MO-injected fish showed normal thrombus initiation but severely impaired thrombus growth, consistent with the mouse knockout phenotypes, and concomitant knockdown of both resulted in spontaneous bleeding. Knockdown of 4 of the 5 novel platelet proteins altered arterial thrombosis, as demonstrated by modified kinetics of thrombus initiation and/or development. We identified a putative role for BAMBI and LRRC32 in promotion and DCBLD2 and ESAM in inhibition of thrombus formation. We conclude that phenotypic analysis of MO-injected zebrafish is a fast and powerful method for initial screening of novel platelet proteins for function in thrombosis.

**Introduction**

Platelets play a central role in hemostasis, adhering to damaged or activated endothelium and aggregating to form a thrombus. Receptor-ligand interactions on the platelet surface regulate these processes. Recent transcriptome and proteome studies of platelets or their precursor cell, the megakaryocyte (MK), have increased our understanding of the complement of proteins present in the human platelet membrane.1-3 Approximately 10 000 genes are transcribed in MKs1,2,4 and less than 1% of these are lineage restricted compared with nucleated blood cell elements and erythroblasts.4 Some of the functionally best-characterized platelet membrane proteins are represented in this small set of transcripts (eg, glycoprotein [GP] IIb), whereas others encode transmembrane proteins whose roles in hemostasis and thrombosis are unknown. Unfortunately, most of the approaches that can be applied to characterize novel genes are of limited use when studying human platelets or investigating large sets of candidate genes. For example, large-scale RNAi screens, commonly used in other cell types, cannot be replicated for platelets because of a combination of factors, including the inability to culture human platelets in vitro or the low efficiency of viral transformation of human hematopoietic stem cells and their progeny.

In this study, we investigated the suitability of zebrafish for functional analysis of novel platelet genes. The zebrafish has recently emerged as an attractive model organism for studying vertebrate hemostasis, as it uniquely combines the advantages of genetic tractability with biologic relevance.5 The coagulation pathways and genetic programs regulating hematopoiesis and vasculogenesis are conserved,6-9 which support the use of zebrafish in the study of human blood diseases. Moreover, zebrafish have nucleated thrombocytes that function in a similar manner to human platelets.10-12 Adhesion and aggregation in response to platelet agonists are comparable,11 and antisera against the human forms of GPIb-a and GPIbIIB are reactive with zebrafish thrombocytes.11

Zebrafish offer several practical advantages over mice for this type of study. First, external fertilization and transparency of zebrafish embryos allow better visualization of early blood-related phenotypes than mice, where development occurs in utero. Second, zebrafish can survive without blood for the first 7 days of life, allowing the function of genes that give lethal phenotypes when knocked out in mammals to be investigated. Finally, knockdown of gene function by antisense morpholino oligonucleotides (MOs) in zebrafish is more suitable to the screening of a large number of

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genes than classic gene knockout in mice\(^1\)\(^3\) and gives an immediate phenotype.

Using a laser-induced thrombosis model to characterize thrombus formation in arteries of zebrafish where known and novel platelet genes were knocked down by MOs, we show that key proteins involved in platelet aggregation and in coagulation in humans, namely, GPIIb and coagulation factor (F) VIII, are also required for normal hemostasis in the zebrafish. We previously identified a series of genes encoding platelet transmembrane proteins that may represent novel regulators of human platelet function\(^2\) and have now used the zebrafish model to conduct the first reverse genetics screen in zebrafish of candidate genes identified in systems biology studies of human blood cell elements.

**Methods**

**Microarray studies and bioinformatics**

Comparative whole-genome expression analysis of the major blood cell elements and in vitro–differentiated MKs and erythroblasts (EBs) was described previously.\(^4\) In summary, natural killer cells, monocytes, granulocytes, B cells, cytotoxic T cells, and helper T cells were isolated by positive selection using magnetic bead technology from peripheral blood from consenting volunteer blood donors of the Cambridge BioResource. In vitro–differentiated MKs and EBs were derived from CD34\(^1\) hematopoietic stem cells obtained from 5 different anonymized cord bloods collected after informed consent in accordance with the Declaration of Helsinki and the guidelines of Eurocord Netherlands.\(^2\)

The transcriptomes of these and human umbilical vein endothelial cells were profiled by microarray and differentially expressed genes were identified by pairwise comparisons.\(^4\) Approval for these studies was obtained from the National Health Service Research Ethics Committee (Huntingdon, United Kingdom). Functional protein domains were obtained from the National Health Service Research Ethics Committee (Huntingdon, United Kingdom). Functional protein domains were predicted using the Eukaryotic Linear Motif resource.\(^4\)

**Confirmation of transcript expression in human platelets**

The expression of candidate genes was validated by quantitative TaqMan real-time polymerase chain reaction (RT-PCR). Total RNA (100 ng) was reverse-transcribed using the reverse transcription protocol (Promega, Madison, WI) and an oligo (dT) primer. After a 1-hour incubation at 42°C, 2 ng cDNA was amplified with template-specific primers using the Mini Opticon real-time PCR system with SYBR green master mix (Bio-Rad, Hercules, CA). Reactions were incubated for 94°C for 10 minutes, and real-time PCR performed more than 40 cycles (94°C for 30 seconds, 52°C for 30 seconds, 72°C for 30 seconds). Data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using a standard curve generated with a dilution series of GAPDH plasmid from which copy number per nanogram of RNA was determined. CD16\(^\text{a}\) cells had the lowest level of GAPDH; therefore, the ratio of GAPDH copies per nanogram (CD16\(^\text{a}\)) to GAPDH copies per nanogram (other cell type) was used to normalize the remaining data.

**Antibody staining of human platelets**

Polyclonal antisera were generated against *Escherichia coli*–produced human recombinant proteins in mice as described previously.\(^2\) cDNAs corresponding to the extracellular domains of human *ANTXR2* (residues 33-318), *BAMBI* (residues 27-153), *DCBLD2* (residues 66-163), *ESAM* (residues 30-247), and *LRRC32* (residues 29-296) were used to produce the corresponding to the extracellular domains of the Cambridge Zebrafish orthologs were predicted by reciprocal BLAST analysis using the Ensembl utilities at http://www.ensembl.org. The criteria for a probable Zebrafish embryo collection

General maintenance, collection, and staging of the zebrafish were carried out according to the Zebrafish Book.\(^{15}\) Embryos were maintained in egg water (60 mg/L Red Sea salts, 2 mg/L methylene blue) until the appropriate

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**Table 1. MO sequences**

| HUGO ID | Zebrafish transcript\(^*\) | MO | Sequence 5'–3' |
|---------|---------------------------|----|----------------|
| ANTXR2  | ENSDART00000085367 | antxr2 atg1 | CGCTCCAGAGATTTTCTTTGGTGACAT |
|         | ENSDART0000007732 | antxr2 sp1 | CGCAGAGTTTGAGCTTGAGAAATAA |
| BAMBI   | ENSDART00000089582 | bambi atg1 | ACCACAGAAACACGGCTGCTCAT |
|         | ENSDART000031181 | bambi sp1 | GATCTCTCTCAGAAACACGAAGAC |
| DCBLD2  | ENSDART00000014159 | dcbld2 atg1 | TGCATAATGCTAAAGTGTCCCGATC |
| ESAM    | ENSDART00000016562 | esam atg1 | GAGGATCTACTGAAAGACAGAAATGA |
| F8      | ENSDART00000059478 | f8 atg1 | CGCAGATTTGGACTGGAATGAAAAA |
| ITGA2B  | ENSDART00000016562 | itga2b atg1 | TTGTTCATCTGCAATACACACCAAGA |
| LRRCS2  | ENSDART00000016562 | lrrc32 atg1 | TCGATACTCCAGAGACAGACAGCCC |
|         | ENSDART00000031181 | lrrc32 sp1 | GACGACAGTTTCTGGACAGATCCAG |

\(^*\)Ensembl identifier.
stage. The approximate stages are given in days post-fertilization (dpf) at
28°C according to morphologic criteria.16

Phenotypic analyses

Morphology. Gross morphology was analyzed by intravital light-field
microscopy. Inspection for spontaneous bleeding was carried out on
unmanipulated larvae 2 to 4 dpf.

Laser-induced thrombosis model. Zebrafish larvae that showed
normal blood circulation at 3 or 4 dpf were anesthetized in Tricaine solution
(300 μM; Sigma-Aldrich, St Louis, MO) for 20 minutes and placed under
the microscope (Leica DMRXA microscope, Wetzlar, Germany; 40×
water-immersion objective). Injury of the caudal artery endothelium was by a
nitrogen-pulse ablating laser (VSL-337; LSI Laser Science, Newton, MA)
and was delivered for 1 to 5 seconds at 3 pulses/second at the maximum
intensity setting. Each larva was injured only once. Images of wild-type and
CD41–green fluorescent protein (GFP) transgenic zebrafish17 were ac-
tained before and after injury to measure thrombus surface
area (TSA) using ImageJ software.18 Briefly, for each image an outline was
drawn around the thrombus (Figure 3B, examples) and the surface area
within calculated automatically. For each experiment, typically 10 to
20 MO-injected larvae were screened per MO with a similar number of
matched sibling controls tested before and afterward, the total experiment
lasting less than 4 hours. Each experiment was repeated at least once using
offspring of different parents.

Statistical analysis of the thrombosis model

For individual experiments, statistical analyses of TTA and TSA were performed
using an unpaired Student t test. For the combined analyses, TTA was modeled
using Cox regression stratifying by experimental day. The severity of the injury
did not significantly affect the model, and so was not adjusted for. Although TSA
is highly skewed, log10 (TSA) is approximately normally distributed; therefore,
log10 (TSA) was fitted with a linear mixed model with (1) fixed effects for larva
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log10 (TSA) was fitted with a linear mixed model with (1) fixed effects for larva
type (control or MO-injected fish) and time after injury (1, 2, 3, or 4 minutes)
and (2) random effects for experimental day and larva within experimental day.
The estimated coefficients were antiloged to transform back to the original scale
(square pixels). Using contrasts, we tested whether the effect of the 2 MOs tested
differed. In most cases, there was no significant difference (P < .05), and so the
MOs were combined.

Results

ANTXR2, BAMBI, DCBLD2, ESAM, and LRRC32 are present in
human platelets and are expressed at higher levels in MKs than
in other blood cell lineages

In a previous study, comparative whole-genome expression analysis of
helper and cytotoxic T cells, monocytes, B cells, NK cells, granulocytes,
EBs, and MKs allowed the identification of genes with up-regulated
expression in MKs compared with all other cell lineages.4 Because
platelets are derived from MKs, we hypothesized that this subset of
genes would provide good candidates for proteins playing a role in
thrombus formation. Analysis of the expression data indicated that
10 314 genes are present in MKs, of which 1035 are up-regulated in
platelets. In Western blots with platelet lysates, a band of expected size
approximately 67 kDa was detected for BAMBI, whereas the others were of higher
molecular weight, most likely due to glycosylation. ANTXR2 was
approximately 67 kDa, DCBLD2 was 85 kDa, and ESAM ran as
2 bands of 40 and 43 kDa (Figure 2 right panels).

Replication of a Glanzmann thrombasthenia phenotype
validates zebrafish as a suitable model organism for platelet
functional studies

To establish whether zebrafish would be suitable for studying
platelet receptor function, we knocked down the gene encoding
GPIIb, aiming to replicate the well-characterized phenotype.19 In
patients with GT (Glanzmann thrombasthenia), platelet aggrega-
tion, but not adhesion, is severely impaired because of loss of
function of integrin GPIIbIIIa. As expected, the GPIIb MO-injected
fish did not display any gross morphologic malformation, nor was
spontaneous bleeding observed (Figure 3Aii). We hypothesized that
any possible defect in thrombocyte aggregation in the fish
might be accentuated by impairment of the coagulation pathway,
and tested this by concurrent knockdown of the gene encoding the
procoagulant protein FVIII. A subset of these double MO-injected
fish bled spontaneously, most commonly in the tail, but intracranial
bleeds were also observed (20% and 3% of larvae, respectively; Figure 3Aiv). Knockdown of the FVIII gene on its own did not
produce a spontaneous bleeding phenotype (Figure 3Aiii).

To analyze the phenotype of the GPIIb MO-injected fish in
more detail, a thrombosis model was developed. This method is
similar to those described in earlier studies using laser injury to

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initiate thrombus formation in zebrafish\textsuperscript{20,21} and mice\textsuperscript{22} and allows qualitative and quantitative differences in thrombus formation between MO-injected fish and controls to be measured. The transparency of zebrafish larvae at this stage allows blood cell attachment to the site of injury and subsequent thrombus formation to be visualized by intravital light microscopy, without intervention. In addition, we used a well-characterized transgenic zebrafish line with fluorescently labeled thrombocytes, where GFP expression is under the control of the CD41 promoter,\textsuperscript{17} to examine the specific contribution of thrombocytes to thrombus formation in control and MO-injected fish.

An ablating laser was used to injure the endothelial cell wall of the caudal artery of 3- to 4-dpf larvae and the TTA and TSA was measured in each case. These endpoints were chosen because they allow distinction between 2 stages of primary hemostasis: thrombus initiation (including endothelial cell activation, thrombocyte tethering, and activation) and thrombus growth (including aggregation and firm adhesion). In control larvae, thrombi could be reproducibly induced (Figure 3Bi), with the first blood cell attaching within 5 to 20 seconds (Figure 3D). Thrombi were occlusive in approximately 10\% of cases, and partial but incomplete lysis was observed after 2 to 10 minutes (data not shown). As expected, TTA was not significantly delayed in GPIIb MO-injected fish, but a significant reduction (>50\%) in TSA compared with controls was observed (\(P<.001\)) (Figure 3D,E left panels). Similarly, loss of FVIII should have little effect on TTA while...
decreasing thrombus size; and, as anticipated, the TSA in FVIII MO-injected fish was only 33% that of controls (P < 0.016) accompanied by a normal TTA (Figure 3D,E right panels). Expression of itga2b and f8 genes in 3- to 4-dpf control embryos and their successful knockdown in GPIIb and FVIII MO-injected larvae were confirmed by RT-PCR (Figure S2).

Finally, analysis of thrombus formation in the CD41-GFP transgenic zebrafish (Figure 3C; Movie S1) showed that thrombi were initiated by thrombocytes, although in a few cases (< 10%) the first cell to attach was not fluorescent (data not shown). The phenotype of GPIIb and FVIII MO-injected transgenic fish was the same as in MO-injected wild-types; and in all cases, the thrombi were composed primarily of thrombocytes (Figure 3C).

Screening by reverse genetics in zebrafish identifies putative roles for BAMBI, DCBLD2, ESAM, and LRRC32 in thrombus formation

To investigate the potential roles of the 4 candidate genes in thrombus formation, we identified the zebrafish ortholog for each gene (Table 2) and designed 2 MOs per gene to act as mutual controls for assessment of the knockdown phenotype in the thrombosis model. The zebrafish ortholog of the control gene, ANTXR2, was similarly assessed. As shown for itga2b and f8 genes, antxr2, bambi, dcbld2, esam, and lrrc32 genes are expressed in 3- to 4-dpf embryos (Figure S2). No transcript was detected in embryos injected with the splice blocking MOs, confirming...
successful knockdown of each gene in the zebrafish (Figure S2). Representative results for TTA and TSA, obtained in MO-injected larvae compared with siblings, from a single experiment are presented in Figure 4A and B. Overall, 42 to 70 larvae were tested for each gene, and the effect of MOs directed at 2 different sites was similar (Table 3). The one exception was ESAM, where just one of the 2 MOs (esam sp1) resulted in degradation of the esam transcript (Figure S2); thus, we lack a corroborating result for this protein. However, the concordance of the ESAM phenotype with mouse knockout data, discussed later, suggests that the result with esam sp1 is specific.

Combined analysis of data from both MOs for each gene revealed significant alterations in cell attachment and/or thrombus growth for all 4 candidate genes but not for the ANTXR2 MO-injected fish (Figure 4C). BAMBI MO-injected fish had a longer TTA with an average increase of 58% \((P < .001)\) and impaired thrombus development where TSA was reduced by 39% \((P = .006)\). The effect of the DCBLD2 knockdown on TTA was ambiguous but resulted in a clear 51% increase in TSA compared with the controls \((P = .013)\). The ESAM MO-injected fish showed normal TTA but increased thrombus size \((P < .001)\). In the LRRC32 MO-injected fish, there was a marginally significant increase in TTA \((30\% \text{ increase}, P = .051)\), whereas TSA was reduced by 29% compared with controls \((P = .034)\). The \(P\) values for individual MOs and combined values are summarized in Table 3.

**Discussion**

The zebrafish has been proposed as a relevant model for hemostasis and thrombosis\(^5,24,25\) and presents several attractive features over
the standard mouse model, the most practical being the transparency of zebrafish embryos, amenability to gene knockdown, and the short time needed to generate and study the MO-injected fish. In the present study, we report on the use of zebrafish for the initial rapid functional analysis of genes derived from postgenomic studies, with a hitherto unknown role in thrombosis.

We chose to validate this approach by knockdown of the zebrafish gene encoding the integrin subunit αIIb (GPIIb), a well-characterized platelet receptor in humans that is deficient in GT patients. The GPIIb MO-injected fish were morphologically identical to controls, concordant with GT patients where only a small proportion of patients display severe spontaneous bleeding.19 Moreover, when we accentuated the GT phenotype by concurrent knockdown of the gene encoding FVIII,7 the coagulation factor perturbed in hemophilia A patients, we observed spontaneous tail and intracranial bleeds, which correlate with increased severity of bleeding observed in rare cases of coexistence of GT and other inherited bleeding diseases19 or in hemophilia patients who have inadvertently used aspirin.26

We next used a laser-induced thrombosis model for more detailed analysis of the GT phenotype. This method allows the kinetics of thrombus formation to be measured in vivo, by observing thrombus initiation and development in real time. We observed that thrombus formation was severely impaired in GPIIb-deficient mice compared with controls, although there was no difference in initial cell recruitment to the site of vascular injury, in accordance with observations made using GT mouse models.27-29 and the well-characterized role of GPⅠbβⅠⅢa in the later aggregation, but not initial adhesion, stages of thrombus formation.90 These observations highlighted the utility of the system to separate the processes of thrombus initiation from growth, not possible by analysis of gross morphology. Moreover, because knockdown of a critical receptor such as GPIIb did not produce a spontaneous bleeding phenotype, it is clear that the laser-induced thrombosis model is a more sensitive and informative tool to test for loss and gain of function.

A potential drawback with zebrafish knockdown experiments is that all cells and tissues are affected; MOs injected at the 1- to 4-cell stage will be present in every cell in the developing fish. Because we therefore cannot assume that the induced phenotype is purely the result of a defect in the function of thrombocytes alone, we selected genes encoding transmembrane proteins, which in humans have up-regulated expression in platelets or MKs compared with other blood cell types, to optimize our chances of identifying novel genes involved in thrombus formation. The zebrafish orthologs of BAMBI, DCBLD2, ESAM, LRRC32, and the control ANTXR2 were knocked down and morphed in the function of thrombocytes alone, we selected genes encoding transmembrane proteins, which in humans have up-regulated expression in platelets or MKs compared with other blood cell types, to optimize our chances of identifying novel genes involved in thrombus formation. The zebrafish orthologs of BAMBI, DCBLD2, ESAM, LRRC32, and the control ANTXR2 were knocked down and morphed in

| Protein/MO | Mean estimate | CI low | CI high | P    | Mean estimate | CI low | CI high | P    |
|------------|---------------|--------|---------|------|---------------|--------|---------|------|
| ANTXR2     |               |        |         |      |               |        |         |      |
| atg1       | 2.6           | ~59.0  | 40.3    | .910 | 15.3          | ~25.5  | 78.3    | .520 |
| sp1        | 45.9          | ~3.0   | 71.6    | .061 | -33.1         | ~60.7  | 13.8    | .137 |
| Comb*      | 21.6          | ~16.0  | 47.0    | .220 | -6.7          | ~35.1  | 34.3    | .706 |
| Bambi      |               |        |         |      |               |        |         |      |
| atg1       | 81.3          | 39.3   | 94.3    | .005 | -56.9         | ~77.9  | ~16.0   | .014 |
| sp1        | 49.0          | 12.6   | 70.2    | .014 | -33.3         | ~54.3  | ~2.6    | .036 |
| Comb*      | 57.5          | 30.3   | 74.1    | ~001 | -39.2         | ~57.0  | ~14.0   | .005 |
| DCBLD2     |               |        |         |      |               |        |         |      |
| atg1       | 46.1          | 6.3    | 69.0    | .028 | 65.8          | 10.4   | 149.1   | .015 |
| sp1        | -41.6         | -162.8 | 23.7    | .270 | 33.1          | ~18.8  | 118.2   | .253 |
| Comb*      | NA            | NA     | NA      | NA   | 51.1          | 9.5    | 108.6   | .013 |
| ESAM        |               |        |         |      |               |        |         |      |
| sp1        | -6.0          | -98.0  | 43.3    | .860 | 129.5         | 46.9   | 259.8   | ~001 |
| sp2        | -8.0          | -68.0  | 30.8    | .740 | 1.5           | -30.1  | 47.3    | .939 |
| Comb*      | -7.0          | -54.0  | 25.3    | .700 | NA            | NA     | NA      | NA   |
| LRRC32     |               |        |         |      |               |        |         |      |
| atg1       | 14.5          | -31.4  | 44.3    | .480 | -31.2         | -52.3  | -0.8    | .045 |
| sp1        | 53.5          | 12.5   | 75.3    | .018 | -25.0         | -54.7  | 24.3    | .262 |
| Comb*      | 29.8          | 0.0    | 50.8    | .051 | -29.3         | -48.6  | -2.7    | .033 |

*Combined analysis using appropriate statistical models for TTA and TSA.

CI indicates confidence interval; and NA, not applicable.

Table 3. Percentage change in TTA and TSA for each MO

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present in MKs, although only 2 have been characterized in platelets; hence, this may be an interesting avenue of research.

We previously showed that the expression of LRRC32 is restricted to platelets and megakaryocytes. As a structural homolog of GP Ibα and GP V, we predicted that the protein may operate as part of a multiprotein complex, perhaps playing a role in adhesion. Here, our combined analysis of LRRC32 MO-injected fish shows that the TTA is normal, although when the effects of each MO were examined individually, the TTA was increased in both cases. For one of the MOs, this increase reached significance; therefore, we do not wish to exclude the possibility of LRRC32 playing a role in thrombus initiation. The TSA result was more straightforward; both MOs produced a defect in thrombus growth. We suggest that LRRC32 could play a role in platelet-platelet and platelet-endothelial cell interactions, which would tie in with the propensity of LRR-rich receptors to form homotypic interactions. Alternatively, LRRC32 might have an as yet unidentified ligand in plasma, endothelial cells, or both, in line with what is known for von Willebrand factor and its interaction with the platelet-specific receptor GP Ibα.

For all of these proteins, our work represents the first study on their putative role in thrombus formation. A recent study, however, has shown that Esam knockout mice form larger thrombi in an in vivo thrombosis model. The ESAM protein is expressed on platelet alpha granules and plays a role in increasing vascular permeability at endothelial cell tight junctions. Thus, it is quite possible that in platelets ESAM functions to destabilize platelet-platelet cell contacts. In agreement with this hypothesis, we found that fish injected with esam sp1 had normal thrombus initiation but dramatically increased thrombus growth and size. Replication of the phenotype observed in mice lends further support to the validity of this zebrafish model.

Knockdown of the zebrafish ANTXR2 ortholog did not significantly alter TTA, nor did it affect thrombus formation in MO-injected fish compared with controls. Because this gene is ubiquitously expressed compared with the other 4, these results show that, as predicted, MK/endothelial-specific gene expression is a strong indicator of a putative function in thrombosis.

The identification of 4 new genes that play a role in thrombus formation, together with the evidence that the proteins are expressed in human platelets, provides enough arguments for these genes to be studied further. On the basis of the functional data gained from this MO-based screen in the zebrafish, we are increasing screening throughput by combining Targeted Induced Local Lesions In Genomes (TILLING) with automated image capture coupled with computer-based pattern recognition of the processes, leading to thrombus formation. This will allow many more genes to be characterized and put us one step closer to understanding the complex networks of interactions that take place within and between platelets and endothelial cells after vessel wall damage. In addition, the generation of genetically modified platelets and endothelial cells, for example, by Cre/Lox-based tissue-specific gene knockout in mice, will allow a more detailed study of the function of these genes in either tissue.

In conclusion, we have demonstrated the suitability of the zebrafish thrombosis model for the functional characterization of novel platelet proteins. This model will facilitate our understanding of the role of novel genes being identified in transcriptional studies of MKs and platelets and also in genome-wide association studies in patients with myocardial infarction and has the potential to contribute significantly to systems biology studies of platelets in health and disease.

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Authorship

Contribution: M.N.O. and I.I.S. designed and performed research, analyzed data, and wrote the paper; N.A.W. and W.H.O. designed research, analyzed data, and wrote the paper; A.W., S.F.G., C.I.J., and A.C. designed and performed research; I.C.M. designed and performed research and analyzed data; J.-J.Z. performed research and analyzed data; S.L.B. and F.D. performed statistical analysis of data; M.S. analyzed data and provided critical reagents; B.d.B. provided bioinformatic support; A.H.G. designed research; D.L.S. designed research and provided laboratory space and reagents; and H.D. designed research and wrote the paper.

A complete list of participating Bloodomics Consortium members appears in the online Supplemental Appendix.

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

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