HHS Public Access

Author manuscript

Am J Kidney Dis. Author manuscript; available in PMC 2024 March 29.

Published in final edited form as:

Am J Kidney Dis. 2019 July ; 74(1): 73–81. doi:10.1053/j.ajkd.2018.12.035.

Transcriptomics of Human Arteriovenous Fistula Failure: Genes Associated With Nonmaturation

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Financial Disclosure: The authors declare that they have no relevant financial interests.

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Abstract

Rationale & Objective: Improving arteriovenous fistula (AVF) outcomes requires better understanding of the biology underlying maturation or failure. Our current knowledge of maturation relies on extrapolation from other vascular pathologies, which does not incorporate unique aspects of AVF remodeling. This study compares the RNA expression of pre-access (native) veins and AVFs with distinct maturation outcomes.

Study Design: Case-control study.

Setting & Participants: 64 patients undergoing 2-stage AVF surgeries at a single center. 19 native veins and 19 AVF samples were analyzed using RNA sequencing (RNA-seq). 58 native veins were studied using real-time polymerase chain reaction; 45, using immunohistochemistry; and 19, using Western blot analysis.

Predictor: RNA expression in native veins and AVFs.

Outcome: Anatomic nonmaturation, defined as an AVF that never achieved an internal diameter ≥ 6 mm.

Analytical Approach: Pre-access native veins and AVF samples were obtained from patients undergoing 2-stage AVF creation. Veins that subsequently matured or failed after access creation were analyzed using RNA-seq to search for genes associated with maturation failure. Genes associated with nonmaturation were confirmed using real-time polymerase chain reaction, immunohistochemistry, and Western blot analysis. In addition, the association between pre-access gene expression and postoperative morphology was evaluated. RNA-seq was also performed on AVFs to search for transcriptional differences between AVFs that matured and those that failed at the time of transposition.

Results: Pro-inflammatory genes (*CSF3R, FPR1, S100A8, S100A9,* and *VNN2*) were upregulated in pre-access veins that failed (false discovery rate < 0.05), and their expression colocalized to smooth muscle cells. Expression of *S100A8* and *S100A9* correlated with postoperative intimal hyperplasia and the product of medial fibrosis and intimal hyperplasia (r = 0.32–0.38; P < 0.05). AVFs that matured or failed were transcriptionally similar at the time of transposition.

Limitations: Small sample size, analysis of only upper-arm veins and transposed fistulas.

Conclusions: Increased expression of proinflammatory genes in pre-access veins appears to be associated with greater risk for AVF nonmaturation.

The biological determinants of arteriovenous fistula (AVF) maturation or failure remain uncertain. Up to 50% of AVFs remodel adversely,[1,2] generating stenotic segments that increase resistance and compromise blood flow. Despite a significant body of histologic data for humans, the field of vascular biology has fallen behind in the discovery of the molecular pathways underlying fistula failure. There is a substantial lag in the application of unbiased "omics"-based approaches compared with other research areas.[3–6] This stagnation has partly contributed to the lack of preventive therapies for AVF complications.

A combination of pre-existing and postoperative factors likely contributes to AVF maturation outcomes. Adequate diameters of the native vein and artery[7–9] and good preoperative arterial function[10] help reach the desired maturation independently of any pre-existing vascular abnormalities such as intimal hyperplasia, medial fibrosis, and microcalcification.[11–15] However, the postoperative factors are related to the vascular response to surgical or biological trauma. We have recently demonstrated that excessive venous fibrosis after AVF creation increases the occlusive potential of intimal hyperplasia and the risk for maturation failure.[15] However, the pre-existing and/or postoperative molecular factors that contribute to AVF morphometry and maturation outcomes are unidentified.

In this work, we applied high-throughput RNA sequencing (RNA-seq) to search for genes associated with greater risk for maturation failure in native veins used for 2-stage AVF creation. The resulting upper-arm AVFs were also analyzed using RNA-seq to look for genes associated with nonmaturation at the time of transposition. We demonstrate the utility of omics approaches in the discovery of the potential mechanisms underlying AVF maturation or failure.

Methods

Study Design

This case-control study was designed to: (1) evaluate whether "pre-existing" gene expression in native (pre-access) veins is associated with subsequent AVF maturation failure, and (2) find differentially expressed genes in remodeled AVFs that had distinct maturation outcomes. Patients with chronic kidney disease who were 21 years or older and scheduled for 2-stage AVF creation surgery (Fig S1) at Jackson Memorial Hospital and University of Miami Hospital were invited to participate in the study.

A single surgeon (M.T.) performed all surgical procedures.[14] We followed the order of AVF preference recommended by the KDOQI (Kidney Disease Outcomes Quality Initiative) clinical practice guideline on hemodialysis adequacy.[16] The surgeon collected vascular samples at 2 times: a segment of the vein used to create the arteriovenous anastomosis during the first-stage surgery and a sample of the juxta-anastomotic area of both mature and failed AVFs during the second-stage transposition or salvage procedure (Figs 1 and S1). Of note, although the native vein and corresponding AVF venous samples are related because they originate from the same individual and same anatomical location, they represent 2 different types of tissues, collected at different time points, that is, before and after the process of AVF maturation. Throughout this article, when we refer to a native vein that matured or failed postoperatively, we mean a vein that generated an AVF that matured or failed, respectively. Anatomic AVF nonmaturation was defined retrospectively as an AVF that never achieved a luminal diameter ≥ 6 mm as determined intraoperatively using intravascular probes.

Veins and AVFs were collected from 106 participants from November 2014 to September 2017 as part of an actively enrolling biorepository. Twenty-eight of these patients had AVF nonmaturation (failed subgroup, n = 28) and were all included in the study. Patients with successful maturation (matured subgroup, n = 36) were then chosen from the repository to have similar representation of baseline characteristics (age range, sex distribution, ethnicity, comorbid conditions, and previous vascular access history) as the failed subgroup (Fig 1). Tissues from 12 patients in each outcome group were selected for gene discovery using RNA-seq (Fig 2A), such that each group included: (1) equal numbers of women and men, (2) similar demographic distribution (age range and ethnicity), and (3) similar clinical history (comorbid conditions and previous vascular access history). Confirmatory analyses of pre-existing gene expression and its association with subsequent failure/maturation included 64 patients. Based on sample availability, 58 samples were analyzed using real-time polymerase chain reaction (PCR), 45 were screened using immunohistochemistry, and 19 were screened using Western blot (Fig 2B; Table S1). Further, 42 patients were included in the assessment of postoperative morphometry (histology) and its correlation with pre-existing gene expression (Table S1).

The study was performed according to the ethical principles of the Declaration of Helsinki. Informed consent was obtained before each surgery. The Ethics Committee and Institutional Review Board at the University of Miami approved the study.

Specimen Collection and Tissue Processing

Native vein and AVF venous samples, 2 to 5 mm in length, were submerged in RNA*later* (Qiagen) immediately after collection, deidentified with a numerical code, and stored at −80°C. The AVF biopsy specimen was taken ~2 cm from the initial anastomosis. If enough tissue was available, a 1- to 2-mm cross-section was fixed in 10% neutral formalin (Sigma-Aldrich) before paraffin embedding and sectioning.

Gene Discovery and Analysis

Total RNA Extraction—Approximately 50 to 60 mg of tissue was ground to a fine powder in a Spex/Mill 6770 (Spex SamplePrep). Total RNA was isolated using Trizol (Thermo Fisher Scientific) and further purified using the E.Z.N.A. Total RNA Kit I (Omega Bio-tek). Item S1 provides detailed methods, including further information on the RNA isolation procedures.

RNA Sequencing—Preparation and sequencing of RNA libraries was carried out in the John P. Hussman Institute for Human Genomics, Center for Genome Technology. Briefly, total RNA was quantified and qualified using the Agilent Bioanalyzer to have an RNA integrity score > 5. Then 500 ng of total RNA was used as input for the Illumina TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero to create ribosomal RNA–depleted sequencing libraries. Each sample was sequenced to more than 40 million raw reads on the Illumina NextSeq500 (see Item S1 for details on processing of raw sequence data).

Differential Gene Expression Analysis—Gene count data were input into edgeR software for differential expression analysis (detailed methodology in Item S1).[17] The false discovery rate was determined by applying the Benjamini-Hochberg multiplicity correction method. Item S2 includes the R codes used in the differential gene expression analyses. RNA-seq data are accessible in NCBI Gene Expression Omnibus through the GEO accession number GSE119296.

Confirmatory Tests in Native Veins

Quantitative Real-Time PCR—Gene expression differences identified using RNA-seq in native veins that matured versus failed postoperatively were confirmed using real-time PCR using TaqMan Gene Expression Assays (Applied Biosystems), as specified in Item S1. Relative gene expression was determined using the ΔΔCT method[18] and normalized with respect to GAPDH expression. Gene expression levels for individual patients were expressed as the $\log_2$ of the fold change (FC) with respect to the average in the matured subgroup.

Protein Isolation and Western Blot—Genes that remained significantly associated with failure after real-time PCR confirmatory tests were further quantified at the protein level. Whole-cell protein lysates were prepared from about 50 to 60 mg of native vein tissue (detailed methodology in Item S1); 25 μg of sample was loaded onto a 12% Tris-glycine sodium dodecyl sulfate polyacrylamide gel and subsequently transferred to a polyvinylidene difluoride membrane (GE Healthcare). Specific proteins were detected using antibodies against calprotectin (S100A8/A9), CSF3R, FPR1, GAPDH, and VNN2 (Item S1). Protein expression levels were normalized with respect to GAPDH.

Immunohistochemistry and Confocal Microscopy—For immunohistochemistry, paraffin sections of pre-access veins were rehydrated, blocked, and treated with 3% hydrogen peroxide (detailed methodology in Item S1). Specific proteins were detected using the above primary antibodies and MCA1815 (Bio-Rad) for CD68. Images were acquired using a VisionTek DM01 digital microscope (Sakura Finetek).

For confocal microscopy, sections were incubated overnight with rabbit anti-S100A9 polyclonal antibodies (diluted 1:100; ab63818; Abcam) and mouse anti-human smooth muscle action clone 1A4 (diluted 1:50; Agilent). After secondary antibodies were applied (Item S1), sections were examined using a confocal scanning laser microscope Zeiss LSM 510 META (Carl Zeiss Microscopy).

Assessment of Postoperative Morphometry

AVF sections (n = 42 of 64 patients included in the study; Table S1) were stained with Masson trichrome for gross histomorphometric analysis. Twenty-two patients were excluded from this analysis because either the real-time PCR native vein expression data were not available (n = 6; Table S1) and/or the AVF sample was insufficient or of low quality (n = 16; Table S1). Intima and media areas were delineated to calculate medial fibrosis (percent area of collagen) and intimal hyperplasia (defined as intima to media area ratio). Medial fibrosis was quantified using ImageJ (National Institutes of Health) and color thresholding methods. Operators blinded to the clinical data (A.P. and J.C.D.) performed digital processing of images and morphometric measurements.

Statistical Analysis

Statistical analyses of tabular data (Item S1) were performed using XLSTAT (Addinsoft) and GraphPad Prism 5.00 (GraphPad Software).

Results

Characteristics of the Experimental Groups

Total RNA was isolated from native veins and juxta-anastomotic AVF samples from 24 patients undergoing surgeries for 2-stage AVF creation (12 patients who had anatomic maturation and 12 who had nonmaturation; Fig 2A). RNA from 5 veins and 5 AVFs (both tissue types from 3 patients and 2 veins and 2 AVFs from separate individuals) did not pass the quality requirements for RNA-seq (RNA integrity score > 5) and differential gene expression analysis (≥80% uniquely mapped reads). After these exclusions, 2 experimental groups were formed to study the pre-existing (ie, before creation of the arteriovenous anastomosis) and postoperative gene expression differences associated with maturation outcomes (Fig 2A). Group I consisted of RNA samples from 19 native veins (11 that matured postoperatively and 8 that failed), while group II included 19 AVF biopsy samples (11 that had matured and 8 that had failed). The matured and failed subsets in both experimental groups were similar in terms of patients' baseline characteristics (Table 1).

Group I Analysis: Gene Expression in Native Veins Associated With Nonmaturation

Group I compared gene expression profiles of native veins that failed versus veins that matured after AVF creation, with the purpose of identifying genetic processes that predisposed for maturation failure. A total of 53,466 distinct RNA features were detected using RNA-seq in the 19 native veins analyzed in this group (Fig 2A). Thirty-six percent of the features corresponded to protein-coding genes; 24%, pseudogenes; 28%, long noncoding RNAs; and 10%, short noncoding RNAs (Fig S2A). As expected, the total expression count

of protein-coding RNAs was significantly higher than the other RNA biotypes in the samples (Fig S2B).

Seven genes were detected in native veins in association with AVF nonmaturation after adjusting for sex-related differences in gene expression (Fig 3; Table 2; Supplementary File 1). Platelet factor 4 (PF4), formyl peptide receptor 1 (*FPR1*), vanin 2 (*VNN2*), S100 calcium-binding protein A9 (*S100A9*), colony-stimulating factor 3 receptor (*CSF3R*), and C-X-C motif chemokine receptor 1 (*CXCR1*) had higher expression (upregulated 5.1- to 11.1-fold) in veins that later failed versus those that matured (Table 2). In contrast, expression of the long intergenic noncoding RNA (lincRNA) AC006262.6 was 3.4-fold lower in veins that failed. *S100A8* (Fig 3; Table 2) was also selected for further confirmatory analyses due to its biological function as dimer partner of *S100A9*[19] and its borderline statistical significance (false discovery rate = 0.056).

Association of these genes with nonmaturation was evaluated using real-time PCR in veins that had been analyzed using RNA-seq and samples from additional individuals (total n = 58 [34 matured and 24 failed]; Fig 2B). matured and failed confirmatory subsets were similar in terms of patients' baseline characteristics (Table 3). Higher RNA expression of *CSF3R, FPR1, S100A8, S100A9,* and *VNN2* was significantly associated with nonmaturation in this larger patient cohort (Fig 4), whereas CXCR1 and PF4 were just outside the significance threshold (Fig S3). We were unable to amplify the lincRNA AC006262.6 using real-time PCR.

A high correlation (Pearson $r \geq 0.80$; $P < 5.0 \times 10^{-14}$) was observed between RNA expressions of the 5 confirmed genes in the cohort of 58 patients (Fig 4F), suggesting that they are either expressed in the same cell type and/or regulated by a common mechanism. Expressions of *CSF3R, FPR1, S100A8, S100A9,* and *VNN2* were not associated with patients' baseline characteristics (Table S2).

Protein levels for the 5 genes confirmed using real-time PCR were quantified in 19 native vein samples from which additional tissue was available (10 that subsequently matured and 9 that failed; Fig 2B). S100A8 and S100A9 protein levels were significantly higher in the failed subgroup (Fig S4). Protein levels of CSF3R were nominally greater in this subgroup, though this did not reach statistical significance (Fig S4), while FPR1 and VNN2 could not be detected using Western blot. Despite the elevated expression of these genes in myeloid cells,[20–22] we found low macrophage counts in native veins from either outcome group (n = 45; Fig S5). Instead, immunohistochemistry for CSF3R and S100A8/A9 (Fig 5) and confocal microscopy for S100A8/A9 (Fig S6) revealed that these inflammatory proteins primarily localized to smooth muscle cells (SMCs).

Given that the association between upregulation of S100A8 and S100A9 and nonmaturation was confirmed at both the RNA and protein levels (Figs 4 and S4), we evaluated whether expression of these genes in native veins correlated with postoperative morphometry in the respective AVF samples (Fig S7). This analysis included 42 individuals from the confirmatory cohort, after excluding 22 patients from whom either the real-time PCR vein expression data (n = 6; Table S1) and/or the postoperative morphometry (n = 16; Table

S1) were not available. We found no associations between pre-existing expressions of S100A8 or S100A9 and postoperative fibrosis (Fig S7). A weak correlation was detected with postoperative intimal hyperplasia ($r = 0.36–0.38$; $P = 0.02$) and with the product of postoperative fibrosis and intimal hyperplasia ($r = 0.32–0.34$; $P = 0.03–0.04$; Fig S7).

Group II Analysis: Gene Expression in AVF Samples Associated With Nonmaturation

Experimental group II had the purpose of evaluating differentially expressed genes in remodeled AVFs that had matured or failed (Fig 2A) to search for postoperative transcriptional signals that distinguished adverse remodeling from successful maturation. All AVF samples were collected within 74 ± 31 (mean ± standard deviation) days after first-stage surgery. A total of 53,360 distinct RNA features were detected using RNA-seq in the 19 AVFs analyzed in this group, with similar representation of RNA biotypes as that observed in pre-access veins (Fig S2).

Only 2 genes were differentially expressed in AVFs that failed compared with AVFs that matured (Fig S8; Table S3; Supplementary File 2), after adjusting for sex-related differences in expression. Butyrylcholinesterase (*BCHE*) and immunoglobulin λ variable 3–1 (IGLV3–1) were downregulated 2.3- and 22.3-fold, respectively, in AVFs that failed versus those that matured (Table S3). A total of 16,911 of the 16,958 genes included in the differential expression analyses had false discovery rates > 0.9 (Supplementary File 2), with only 47 genes separating from the x-axis in the volcano plot representation of gene expression differences (Fig S8). These results indicate that at the time of AVF sample collection, when maturation-associated remodeling is complete, tissues from the 2 outcome groups are transcriptionally similar.

Discussion

The uniqueness of AVF remodeling illustrates the need for omics methodology to uncover the biological mechanisms associated with AVF outcomes. In this pioneering work, we used high-throughput transcriptomic analyses to: (1) identify a unique inflammatory fingerprint in SMCs of native veins from humans that is associated with AVF nonmaturation, and (2) demonstrate that remodeled AVFs that matured or failed are transcriptionally similar at the time of transposition.

The identification of conditions in the native vein that influence subsequent AVF outcomes has been a challenging task. We and others have shown that the severity of intimal hyperplasia and fibrosis in native veins does not increase the likelihood of nonmaturation.[13–15,23] The role of pre-existing vascular inflammation has also been dismissed due to the scarcity of immune cell infiltration in pre-access veins.[24] This work confirms the small number of infiltrating macrophages in native veins, but reveals a molecular signature of inflammatory genes (*CSF3R, FPR1, S100A8, S100A9,* and *VNN2*) upregulated in SMCs that associate with later nonmaturation. After initially picking these genes up using whole transcriptome RNA-seq profiling, we confirmed that higher RNA expression of these genes in pre-access veins was associated with failure in a cohort of 58 patients. Quantification of protein expression was only possible for CSF3R, S100A8, and S100A9, while FPR1 and VNN2 will require more sensitive techniques due to lower protein expression levels.

Interestingly, these genes are usually regarded as markers of myeloid cells.[20–22] However, ectopic expression has been reported in endothelial cells and SMCs in the setting of inflammation.[25–28] All these genes have been implicated in the activation of NF-κB pathways and of the NADPH oxidase complex.[29–33] The inflammatory stimulus leading to their expression in SMCs of pre-access veins may be related to CKD or to hemodialysis therapy initiation before AVF creation. However, their upregulation in veins that fail to mature suggests the existence of additional patient-specific triggers, such as genetic background or previous trauma to the vessel.

Unlike the lack of association between pre-existing venous morphometry and maturation outcomes,[13–15,23] our group has demonstrated that excessive postoperative medial fibrosis and the product of fibrosis and intimal hyperplasia are predictive of AVF failure.[15] Calprotectin is a heterodimer of S100A8 and S100A9, with known inflammatory and profibrotic properties[34] and the only factor confirmed at both RNA and protein expression levels in this study. Given that this is a secreted protein with potential paracrine effects on SMCs, fibroblasts, and myofibroblasts in the wall, we tested the association between expression of the calprotectin genes in pre-access veins and postoperative morphometry. We found no association between S100A8 or S100A9 expression and postoperative AVF fibrosis. Instead, we revealed a weak correlation between expression of the calprotectin genes and postoperative intimal hyperplasia and the product of medial fibrosis and intimal hyperplasia.

Regardless of the final maturation outcome, a vein undergoes a profound cellular and structural transformation as it becomes an AVF and adapts to the conditions of arterial circulation.[35] Surprisingly, our data demonstrated that remodeled AVFs that matured or failed are transcriptionally similar at the time of fistula transposition. This indicates that most transcriptional changes that determine maturation outcomes already exist in the pre-access vein and/or occur early during the maturation period. *BCHE* was the only potentially relevant gene detected by using RNA-seq at the time of second-stage surgery, showing lower RNA expression associated with nonmaturation. Interestingly, a common *BCHE* polymorphism associated with reduced enzymatic activity has been identified as a risk allele for coronary artery disease and in-stent restenosis.[36,37]

Our study demonstrates the utility of omics techniques to identify to date unknown pathways associated with adverse AVF outcomes. Two-stage AVFs provide an added advantage, that is, the opportunity to collect samples of not only the native vein used for fistula creation, but also of the resulting AVFs (both matured and failed) at the time of transposition or salvage procedure. Given the availability of both types of tissues from the same individuals, future work can potentially recreate the vein-to-AVF transformation at the molecular level through the analysis of longitudinal changes in gene expression and identify the master regulators of such transformation.

In conclusion, by combining unbiased transcriptomics technology and well-characterized clinical samples, we aimed to speed the discovery of potential preventive targets to reduce AVF nonmaturation. The limitations of this work include a small sample size, analysis of only upper-arm veins and transposed fistulas, the potential contribution of blood

contaminants to the gene expression analyses, and the possibility that the findings are not generalizable to the broader hemodialysis population or to nontransposed AVFs. Our confirmatory experiments demonstrated the SMC localization of the proinflammatory genes of interest, thus raising questions about the current concept of native vein inflammation. Additional experiments and omics techniques will help expand the mechanistic implications of the present findings, as well as identify new genes involved in AVF remodeling and its complications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

We acknowledge the statistical assistance of Marie J.M. Seignon at the John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL.

Support:

This study was supported by the National Institutes of Health (NIH) grant R01DK098511 to Drs Salman and Vazquez-Padron. The NIH had no role in the study design; collection, analysis, and interpretation of data; writing the report; or the decision to submit the report for publication.

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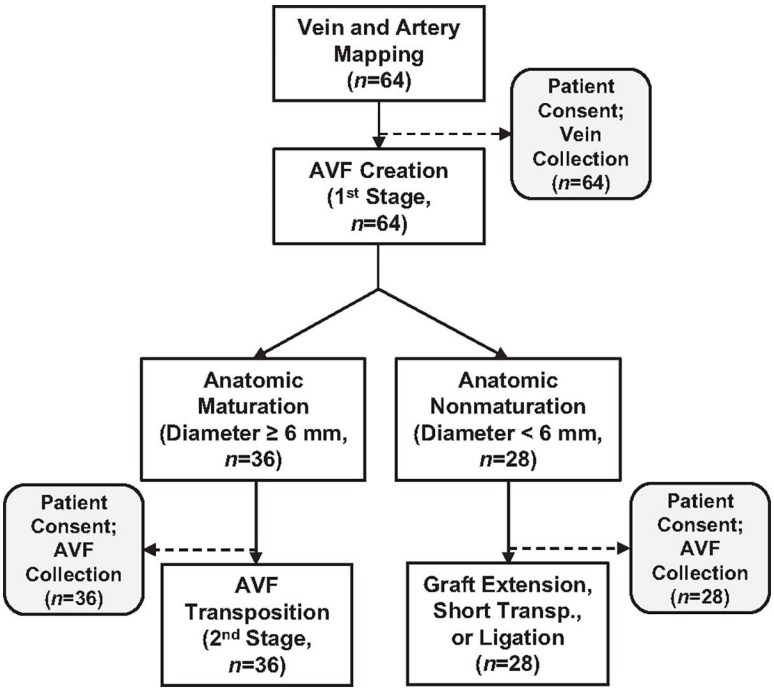

Figure 1.

Flow diagram of surgical procedures and sample collection. The chart illustrates the flow of surgical procedures performed on arteriovenous fistulas (AVFs) that matured and failed and the times of sample collection.

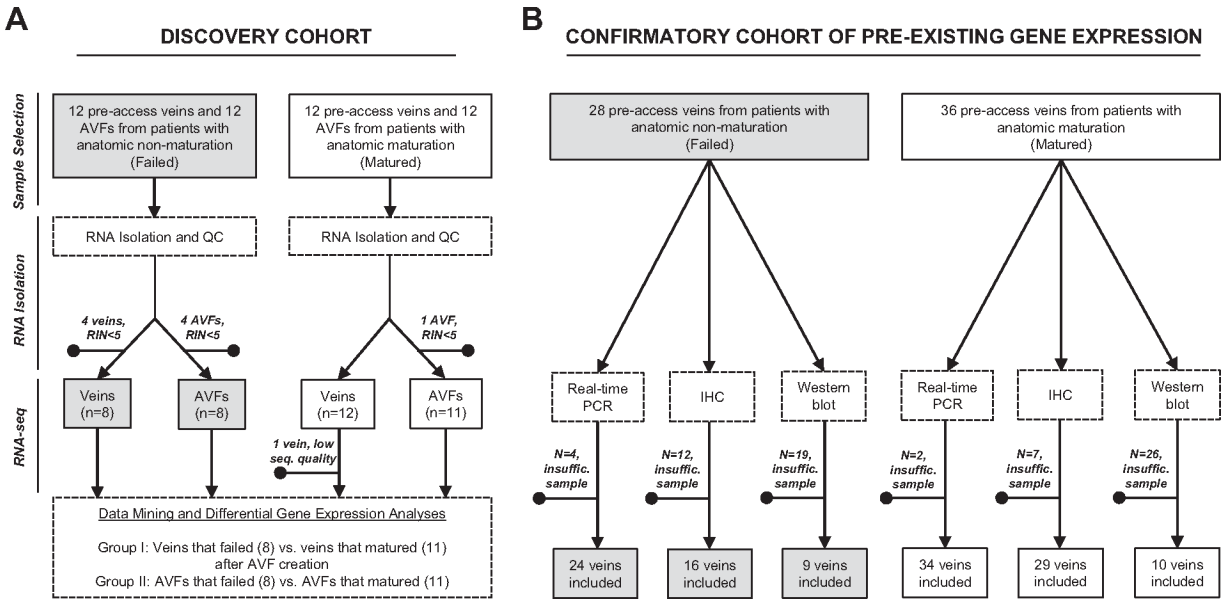

Figure 2.

Sample selection for RNA sequencing (RNA-seq) and confirmatory analyses. (A) Total number of veins and arteriovenous fistulas (AVFs) selected for RNA-seq from each maturation outcome and exclusion criteria. Nine RNA samples did not pass the quality control (QC) requirements for RNA-seq (RNA integrity score [RIN] > 5), and 1 additional vein generated low sequence quality (<80% uniquely mapped reads). After these exclusions, the bottom box indicates the number of samples compared in each experimental group in the differential expression analyses. (B) Total number of veins included in the confirmatory analyses of pre-existing gene expression differences between veins of distinct maturation outcomes. The confirmatory analyses included the veins analyzed in (A) the discovery cohort and were prioritized based on sample availability in the following order: (1) real-time polymerase chain reaction (PCR), (2) immunohistochemistry (IHC), and (3) Western blot.

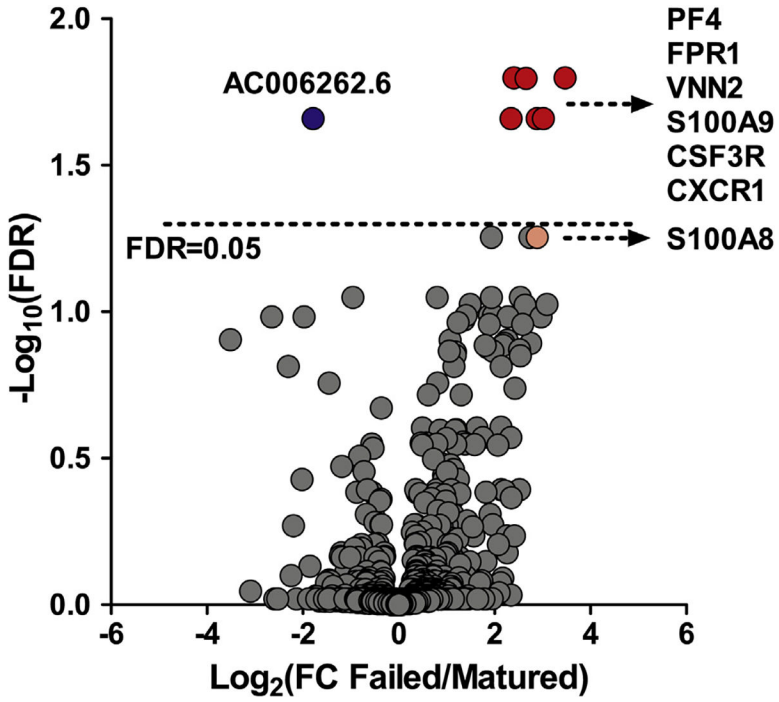

Figure 3.

Genes associated with maturation failure in native veins as determined using RNA sequencing. Volcano plot representation of differentially expressed genes in native veins in association with arteriovenous fistula maturation outcomes. Change in gene expression is presented as fold change (FC) in veins that failed (n = 8) with respect to veins that matured (n = 11) postoperatively. The dashed line indicates the cutoff for a false discovery rate (FDR) of 0.05.

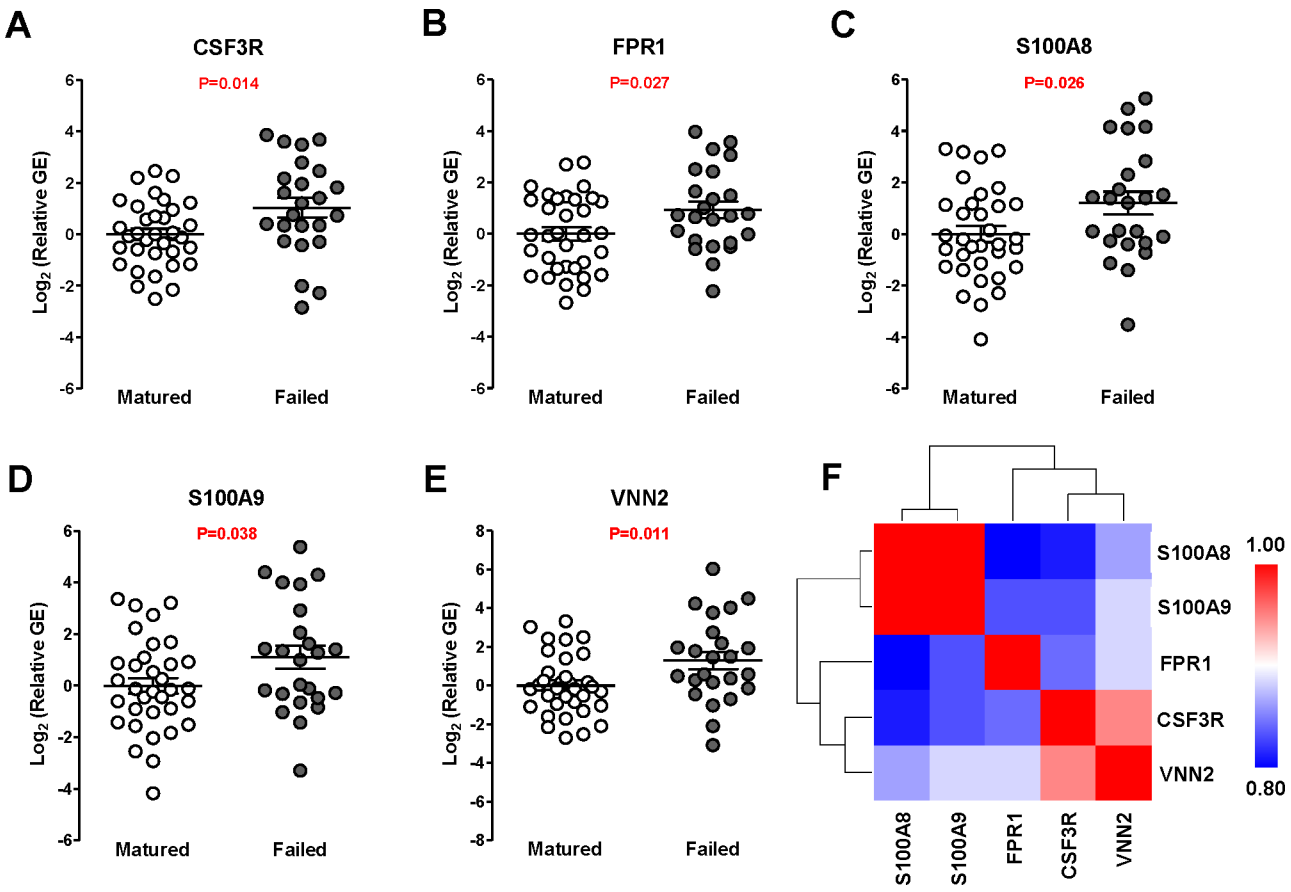

Figure 4.

Genes associated with maturation failure in native veins used for arteriovenous fistula creation. (A-E) Confirmation of gene expression (GE) differences between veins that matured (n = 34) and failed (n = 24) after access creation using real-time polymerase chain reaction. Bars within each group indicate the mean and standard error of the mean (SEM). (F) Pearson correlation matrix of relative GE for differentially expressed genes in the confirmatory cohort. $P < 5.0 \times 10^{-14}$ for each of the correlation tests.

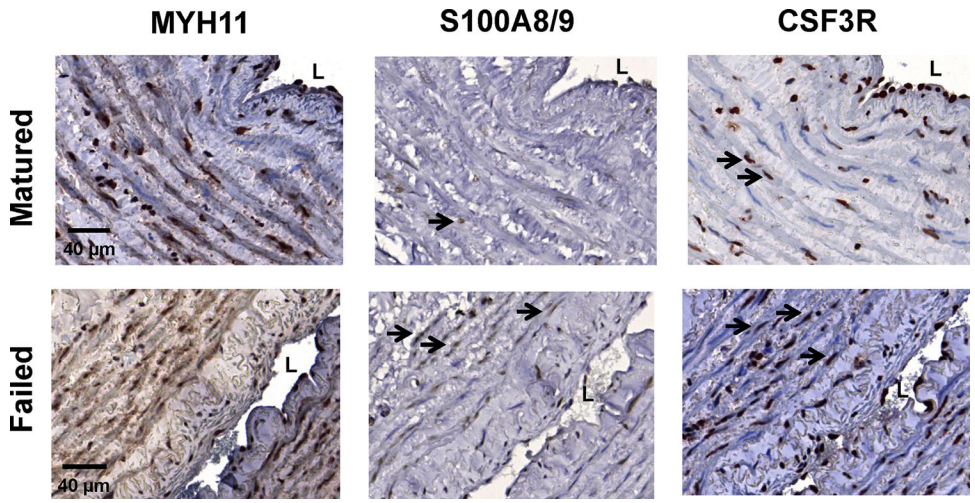

Figure 5.

Expression of proinflammatory proteins in smooth muscle cells (SMCs). Representative immunohistochemistry images of the SMC marker myosin heavy chain 11 (MYH11) and the proinflammatory proteins calprotectin (S100A8/A9) and colony-stimulating factor 3 receptor (CSF3R) in cross-sections from veins that matured and failed after access creation. Arrows indicate examples of S100A8/A9− and CSF3R-positive SMCs. Abbreviation: L, lumen.

Table 1.

Baseline Characteristics of the RNA Sequencing Cohorts

Characteristic	Matured	Failed	*P*
Group I: native veins			
No. of patients	11	8	
Vein diameter, mm	4.0 [4.0–4.0]	4.0 [4.0–4.0]	0.9
Age, y	57.9 ± 17.1	62.4 ± 14.7	0.6
Female sex	5 (45%)	5 (63%)	0.6
Non-Hispanic black[a]	8 (73%)	5 (63%)	0.9
Diabetes	5 (45%)	6 (75%)	0.4
CAD	5 (45%)	3 (38%)	0.9
Previous AVF	1 (9%)	2 (25%)	0.5
Group II: AVFs			
No. of patients	11	8	
AVF diameter, mm	8.0 [7.0–8.0]	5.3 [4.1–5.5]	<0.001
Age, y	56.9 ± 1 7.4	60.3 ± 13.1	0.7
Female sex	5 (45%)	4 (50%)	0.9
Non-Hispanic black[a]	7 (64%)	4 (50%)	0.7
Diabetes	6 (55%)	6 (75%)	0.6
CAD	5 (45%)	4 (50%)	0.9
Previous AVF	1 (9%)	2 (25%)	0.5
Time interval, d[b]	74.7 ± 30.2	72.3 ± 33.9	0.9

Note: Values for continuous variables are given as median [interquartile range] or mean ± standard deviation.

Abbreviations: AVF, arteriovenous fistula; CAD, coronary artery disease.

[a]Patient's ethnicity is Hispanic or non-Hispanic black.

[b]Refers to the wait time between the first- and second-stage surgeries (when the AVF sample was collected).

Table 2.

Genes Associated With Maturation Failure in Pre-Access Veins as Determined Using RNA Sequencing

Gene Symbol	Gene Name	Chromosomal Location	Log_2(FC)	Log_2(CPM)	*P*	FDR
PF4	Platelet factor 4	4q13.3	3.47	1.58	1.33×10^{-06}	0.016
FPR1	Formyl peptide receptor 1	19q13.41	2.40	3.02	1.82×10^{-06}	0.016
VNN2	Vanin 2	6q23.2	2.66	2.82	2.73×10^{-06}	0.016
S100A9	S100 calcium binding protein A9	1q21.3	2.88	5.10	6.32×10^{-06}	0.022
CSF3R	Colony-stimulating factor 3 receptor	1p34.3	2.34	3.95	6.58×10^{-06}	0.022
AC006262.6	Uncharacterized lincRNA	19q13.32	−1.78	−0.16	7.99×10^{-06}	0.022
CXCR1	C-X-C motif chemokine receptor 1	2q35	3.02	1.66	8.76×10^{-06}	0.022
S100A8	S100 calcium-binding protein A8	1q21.3	2.89	3.85	3.18×10^{-05}	0.056[a]

Note: Change in gene expression is expressed as $\log_2$ of the fold change in the failed subgroup with respect to the matured subgroup. Expression levels are expressed as $\log_2$ of counts per million.

Abbreviations: FDR, false discovery rate; lincRNA, long intergenic noncoding RNA.

[a]Despite an FDR > 0.05, S100A8 was selected for further confirmatory analyses due to its biological function as dimer partner of S100A9 and its borderline statistical significance.

Table 3.

Baseline Characteristics of the Native Vein Confirmatory Cohort

Characteristic	Matured	Failed	*P*
No. of patients	34	24	
Vein diameter, mm	4.0 [4.0–4.0]	4.0 [4.0–4.0]	0.2
Age, y	53.4 ± 15.7	58.4 ± 15.4	0.2
Female sex	15 (44%)	13 (54%)	0.6
Non-Hispanic black[a]	20 (59%)	16 (67%)	0.6
Diabetes	16 (47%)	13 (54%)	0.8
CAD	7 (21%)	9 (38%)	0.2
Previous AVF	4 (12%)	5 (21%)	0.5

Note: Values for continuous variables are given as median [interquartile range] or mean ± standard deviation.

Abbreviations: AVF, arteriovenous fistula; CAD, coronary artery disease.

[a]Patient's ethnicity is Hispanic or non-Hispanic black.