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Pathway-driven rare germline variants associated with transplant-associated thrombotic microangiopathy (TA-TMA)

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ZZ performed the statistical analysis, interpreted the data, and wrote the manuscript. WH performed the bioinformatics analysis, interpreted the data, and critically revised the manuscript. QW performed the statistical analysis. ST and JL performed the bioinformatics analysis. PB and RSM created the genetic pathways and critically revised the manuscript. CIA, CC, SS, VAK, JD and PJM interpreted the data and critically revised the manuscript. PKB designed the research and critically revised the manuscript. AL designed the research, performed the statistical analysis, interpreted the data, and wrote the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abstract

The significance of rare germline mutations in transplant-associated thrombotic microangiopathy (TA-TMA) is not well studied. We performed a genetic association study in 100 adult TA-TMA patients vs. 98 post-transplant controls after matching by race, sex, and year. We focused on 5 pathways in complement, von Willebrand factor (VWF) function and related proteins, VWF clearance, ADAMTS13 function and related proteins, and endothelial activation (3,641variants in 52 genes). In the primary analysis focused on 189 functional rare variants, no differential variant enrichment was observed in any of the pathways; specifically, 29% TA-TMA and 33% controls had at least 1 rare complement mutation. In the secondary analysis focused on 37 rare variants predicted to be pathogenic or likely pathogenic by ClinVar, Complement Database, or REVEL in-silico prediction tool, rare variants in the VWF clearance pathway were found to be significantly associated with TA-TMA (p=0.008). On the gene level, *LRP1* was the only one with significantly increased variants in TA-TMA in both analyses (p=0.025 and 0.015). In conclusion, we did not find a significant association between rare variants in the complement pathway and TA-TMA; however, we discovered a new signal in the VWF clearance pathway driven by the gene *LRP1* among likely pathogenic variants.

Keywords

Thrombotic Microangiopathies; Complement System Proteins; von Willebrand Factor; Genetic Association Studies; Hematopoietic Stem Cell Transplantation

Introduction

Thrombotic microangiopathy (TMA) is a rare but devastating hematologic complication after hematopoietic cell transplantation (HCT) that manifests as uncontrolled microangiopathic hemolytic anemia (MAHA) with microvascular thrombosis.[1] Affecting approximately 12% of HCT patients,[2] transplant-associated TMA (TA-TMA) has a reported case fatality rate of 30–50% as well as increased risk of acute and chronic kidney diseases.[3–6] In recent biomarker studies, elevations in alternative complement factors SC5b9 and Ba as well as endothelial injury markers ANG-2 and ST2 have been reported among TA-TMA patients.[7–12] To the contrary, significant variations among antigen levels of von Willebrand Factors (VWF) and a disintegrin and metallopeptidase with thrombospondin type 1 motif 13 (ADAMTS13) have not been observed *in vitro*.[5,13,14]

Several rare variant genetic association studies have been performed using targeted next generation sequencing (NGS) directed at complement genes. While Jodele et al. showed significantly increased rare variant burden in many complement genes among 34 pediatric and young adult TA-TMA patients, Gavriilaki et al. showed a more attenuated burden among 40 adult TA-TMA patients.[15,16] Furthermore, neither study examined additional pathways outside of complement genes or used rare variant-specific genetic association collapsing statistical tests.[17] Leveraging existing biological knowledge from the previous biomarker

studies, we performed a hypothesis-driven rare variant association study in 100 adult TA-TMA patients vs. 98 post-transplant controls without TMA. Specifically, we examined the association between TA-TMA development and each of 5 discrete pathways involving complement factors, von Willebrand factor (VWF) function and related proteins, VWF clearance, ADAMTS13 and related proteins, and endothelial activation.

Methods

Study population and case/control sampling

We conducted a nested case-control study using pre-transplant germline DNA samples from patients undergoing allogenic HCT at the Fred Hutchinson Cancer Research Center (FHCRC) from 2006 to 2015. We selected 100 patients with a diagnosis of TA-TMA using clinical and laboratory case definitions described in our previous studies.[12,18,19] This definition most closely mimics the Cho criteria[20] and requires consecutive laboratory criteria for microangiopathic hemolytic anemia plus no other obvious causes for TMA through clinical chart review (Supplemental Table 1). We performed incidence density sampling to randomly select 100 non-TMA controls after allogeneic HCT after frequency-matching by race, sex, and year of HCT. To avoid over-matching, we did not match controls based on other pre- or post-transplant characteristics.

Sequencing and joint variant calling

We performed whole exome sequencing at the Yale Center for Genome Analysis. The sequencing was performed using Illumina NovaSeq for germline variant detection at an average 40x depth where the obtained sequencing had >93% coverage at 15x depth on a per-sample basis. Sequence reads were mapped to the human reference GRCh38/hg38, followed by de-duplication and Base Quality Score Recalibration to generate analysis-ready BAM files.

We chose the candidate genes at the conception of the study based on biological functional relatedness and classified into discrete pathways in relation to complement (18 genes), ADAMTS13 and mimics (10 genes), VWF function and related proteins (7 genes), VWF clearance (10 genes), and endothelial activation (7 genes) (supplemental Table 2). Specific gene boundaries within each of the pathways were defined using the RefSeqGene database +/− 10 kilobases.[21] The mean sequencing depth of these 52 genes was ~60x and only one gene (*CFB*) has mean sequencing depth <15x (supplemental figure 1).

Joint genotyping for single-nucleotide variants (SNVs) and insertion/deletions (indels) was performed using VarScan2 v2.4.4 germline tool.[22] The VCF files underwent additional quality control (QC) to filter out variants that had genotype quality (GQ) <30, allele depth (DP) <10, or missing rate >5%. Furthermore, filtering of heterozygous calls required an allelic balance between 25% and 75%, and low-quality SNV sites near indels were deleted. To avoid mismapping due to homologs and paralogs (i.e. pseudo-genes), we extracted the sequence of ±25 bp flanking region of each variant sites and searched the rest of hg38 genome by blastn command of BLAST+ 2.13.0.[23] Variants with flanking region highly similar (>90% identity) to another region of genome were further removed from analysis.

The relatedness of individuals was inferred by KING,[24] and individuals with relatives up to second degree were removed. The final variants were annotated using ANNOVAR[25] and ensemble variants effect predictor (VEP).[26] We performed principal component analysis (PCA) on common variants to examine the population genetic structure and ensure there was no systemic population heterogeneity between cases and controls.

Rare variant selection

After joint variant call and QC, variants were further enriched by applying additional filters to reduce the neutral background signal.[17] Specifically, we kept only known variants with minor allele frequency (MAF) <1% from exome reference databases (GnomAD[27], ExAC[28], 1000G[29]) or novel variants with internal MAF <5% in controls. We further removed likely non-functional variants (i.e. synonymous, intronic) to keep only high- or moderate-impact variant class based on VEP[30] variant consequence as defined by sequence ontology, including stop gained, stop lost, frameshift, inframe deletion, inframe insertion, coding sequence, start lost, protein altering, splice acceptor, and splice donor. To determine pathogenicity (secondary analysis), we reviewed variants previously reported to be *pathogenic or likely pathogenic* from the ClinVar[31] or the Complement Database.[32] For the remaining missense variants, we used the REVEL ensemble in-silico tool to predict their pathogenicity where a REVEL score of >0.644 was considered to be *supporting evidence for pathogenic*.[33]

Statistical analysis

For genetic association testing, rare variants meeting the filtering and enrichment criteria were aggregated by each of 5 genetic pathways and tested for association using the sequence kernel association test (SKAT).[34] As the study was matched on race and sex, additional covariate adjustment for population stratification or sex was not performed. The association between TA-TMA and each of 5 genetic pathways was tested before (primary analysis) and after in-silico prediction of pathogenicity (secondary analysis). In exploratory analyses, we also examined the association between TA-TMA and individual genes, as well as subgroup analysis based on antecedent clinical conditions. All statistical analyses were performed using R 4.0.2 (Vienna, Austria).

Results

Study population

From 2006 to 2015, 2,145 patients at FHCRC underwent allogeneic HCT and 192 developed TA-TMA.[12] From this cohort, 100 TA-TMA cases and 100 non-TMA matched controls who had first-ever allogeneic HCT were selected. After whole exome sequencing, joint variant calling, and quality control filtering, 100 cases and 98 controls remained in the analytic dataset. One patient was excluded due to low quality sequencing and one patient was excluded due to close kinship to another control.

Cases and controls had similar distributions of age, sex, race, year of HCT, prior autologous HCT, underlying disease, conditioning regimen, and GVHD prophylaxis (Table 1). Specifically, there were 82% White, 6% Black, 8% Asians, and 4% other ancestries

among TA-TMA cases and 82% White, 4% Black, 9% Asian, and 4% Other among non-TMA controls. Females represented 47% and 46% of cases and controls, respectively. The two groups were numerically different for the distribution of donor matching and statistically different for the development of acute GVHD post-transplant ($p<0.01$). Grade 3–4 GVHD occurred in 50% of TMA cases but only 6% of non-TMA controls. There was no systemic population genetic heterogeneity between cases and controls based on the distribution of top principal components (supplemental Figure 2).

Rare variant calling and enrichment

Among 3,641 variants in 52 genes from the 5 genetic pathways, 1,426 variants passed the QC, and 751 variants satisfied the rare variant criterion (Figure 1). Of these, 189 variants were missense, nonsense, frameshift, or splicing mutations, 37 variants had either high-confidence loss of function or a missense variant that was previously reported to be likely pathogenic in the ClinVar or the Complement Database or with a REVEL score supportive of pathogenicity. Detailed information on unique variants and allele counts stratified by functional status and pathway/gene are shown in supplemental Tables 3 and 4.

Rare variant association testing on a pathway level

The primary analysis of pathway-specific rare variant association testing is shown in Table 2 and Figure 2. In the complement pathway, 29% of TA-TMA patients vs. 33% controls had at least 1 rare variant. The allele count was 36 in the TA-TMA cases and 39 in the controls, with no significant association observed in comparison tests ($p=0.43$). In the ADAMTS13-related pathway, 16% of TA-TMA patients vs. 10% controls had at least 1 rare variant, with 19 alleles in TA-TMA cases vs. 11 in controls. The association test in this gene set was marginally significant ($p=0.047$). In the VWF clearance pathway, 37% of TA-TMA patients vs. 34% of controls had at least 1 rare variant with allele counts of 41 vs. 42. There was no significant association in aggregation test ($p=0.068$). Finally, both the proportion of patients with at least one rare variant and the overall allele counts were evenly distributed between TA-TMA cases and controls for the remaining 2 pathways (VWF function and related proteins and endothelial activation).

We performed a secondary analysis focused on the 37 high-confidence loss-of-function and likely pathogenic or pathogenic mutations (Table 2). Similar to the primary analysis, we did not observe any enrichment in TA-TMA cases in genes representing the complement pathway (6 variants in TA-TMA cases vs. 9 in controls). In the VWF clearance pathway, although we observed a similar number of alleles in cases ($n=10$) and controls ($n=11$), the differential distribution of variants in specific genes led to significant associations ($p=0.008$). Notably, we did not find a significant association within the ADAMTS13-related pathway in the post-*in silico* analysis.

Rare variant association testing on a gene level

To further investigate the contribution of individual genes within each pathway, we performed exploratory association testing for variant counts in individual genes. In the primary analysis, variant counts in 3 genes were associated with TMA (Table 3). Among them, 2 belonged to the VWF clearance pathway and 1 to the ADAMTS13-related pathway.

The *LRP1* gene had 15 variants in TMA cases vs. 7 in controls (p=0.025). The *PRTN3* gene had 5 variants at a single site in TMA cases vs. 0 in controls (p=0.014). The *CLEC4M* gene had 0 variants in TMA cases vs. 3 variants at a single site in controls (p=0.035), suggesting a possible protective effect. Most other genes showed no statistically significant differences in the distribution of rare variants in cases and controls. When we conducted the same analysis on variants predicted *in silico* to be pathogenic, only *LRP1* (5 variants in cases vs. 2 variants in controls) and *CLEC4M* (0 variants in cases vs. 3 variants in controls) retained a significant association (Table 4). Most of the variants in *PRTN3* did not meet the threshold for pathogenicity. Supplemental Table 5 summarizes the distribution of all variants within the VWF clearance pathway.

Exploratory subgroup analysis

We performed an exploratory subgroup analysis to examine whether the pattern of rare variant distribution differed according to the type of clinical condition underlying the development of TA-TMA (supplemental Figure 3). Based on clinical observations made in our previous study,[12] we sub-divided 100 TA-TMA cases into TMA with preceding acute GVHD (n=56), TMA with preceding systemic infection or diffuse alveolar hemorrhage (DAH) (n=26), or TMA without other post-transplant complications (i.e., idiopathic/drug-related TMA) (n=18). For patients with multiple concurrent antecedent conditions, a ranking order of GVHD followed by infection/DAH followed by idiopathic/drug-related was used. Based on this classification, *LRP1* and *PRTN3* gene variants appeared to be over-represented in TMA patients with antecedent infection/DAH instead of those with antecedent GVHD or non-TMA controls. The allele frequencies were 12% (6/26) in TMA/infection vs. 5% (6/56) in TMA/GVHD vs. 5% (7/98) in controls for *LRP1* and 6% (3/26) in TMA/infection vs. 1% (1/56) in TMA/GVHD vs. 0% (0/98) in controls for *PRTN3*. To further ensure the observed genetic association above was related to TA-TMA and not severe GVHD, we repeated the collapsing rare variant analysis for patients with severe grade 3–4 GVHD (n=56) vs. those with no/mild grade 1–2 GVHD (n=142) regardless of their TA-TMA status. In this sensitivity analysis, neither *LRP1* (5% vs. 6%, p=0.41) nor *PRTN3* (1% vs. 1%, p=0.85) were associated with severe GVHD (supplemental Table 6).

Discussion

In this matched case-control genetic association study utilizing samples from 198 adult allogeneic HCT recipients, we evaluated the impact of rare germline variants in 5 *a priori* designated biological pathways on the development of TA-TMA. Contrary to our initial hypothesis, we did not detect germline rare variant associations between TA-TMA and genes from the complement pathway. In contrast, we found unexpected signal within the VWF clearance pathway both before and after *in-silico* prediction of pathogenicity, particularly in the *LRP1* gene among TA-TMA patients with preceding infections.

The results of the current study should be interpreted in the context of two previous genetic studies. In the first, Jodele et al. used NGS to compare 34 TA-TMA patients versus 43 non-TMA controls among pediatric and young adult allogeneic transplant recipients.[35] The authors found 42 synonymous, missense, nonsense, or splice site variants distributed in 26

patients, where 22/34 (65%) of TA-TMA patients had at least 1 deleterious or benign rare variant compared with 4/43 (9%) of control patients. In the second study, Gavriilaki et al. used NGS to compare 40 TA-TMA patients versus 40 non-TMA controls (and 18 healthy donors) among adult allogeneic HCT recipients over age 19.[36] The authors found 138 novel/rare variants (including many intronic) and 612 exonic/splicing variants (including many common alleles). The authors reported a significantly increased number of rare variants per TA-TMA patient in certain complement genes (*CD46*, *CFH*, and *CFI*) compared to healthy donors.

Our work utilized a complement pathway gene set that was comparable to those utilized in prior studies as well as those employed at the Mayo Clinic reference laboratory[37] and in a major complement database (Supplemental Table 7).[32] Because we employed whole exome sequencing instead of a targeted NGS approach and had a larger sample size, we detected significantly more variants than found in previous studies. However, these variants were evenly distributed between cases and controls. Specifically, 33% of non-TMA control patients had at least 1 rare variant in the complement pathway and 8% of them carried a deleterious variant according to ClinVar *in-silico* prediction. This is comparable to a previous study by Chaturvedi et al. where 23% of normal controls were found to have at least 1 rare mutation in the complement pathway[38]. Based on these findings, germline mutations in complement genes alone are likely insufficient for making a diagnosis of TA-TMA.

Several explanations could account for differences in the frequencies of rare variants in complement genes between our study and previous studies. First, adult TA-TMA patients may differ from pediatric TA-TMA patients, as the majority of the former undergo allogeneic HCT for hematologic malignancies, while many of the latter are often treated for hereditary genetic or immune diseases. Second, the studies differ in their TA-TMA case definitions. Given the lack of a gold standard, groups such as the International Working Group (IWG)[39] and Blood and Marrow Transplant Clinical Trials Network (BMT-CTN)[40] have proposed different diagnostic criteria, and other definitions of TA-TMA are commonly used in HSCT clinical research.[41,42] The incidence of physician/provider diagnosis of TA-TMA reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) was only 3% among 23,665 allogeneic HCT adult and pediatric patients from 2008 to 2016, compared to 12% in our recent systemic review and meta-analysis.[2,43] Differences in the sequencing methods and bioinformatics analyses could also contribute to the variation in results.

In the current study, *LRP1* was the only gene significantly enriched in TA-TMA cases before and after application of *in-silico* pathogenicity prediction. It is intriguing that this signal within the *LRP1* gene was accentuated in TMA patients with prior systemic infection or DAH[44,45] but not in the TMA patients with preceding acute GVHD. The *LRP1* gene encodes a low-density lipoprotein (LDL) receptor related protein that contributes to the clearance of VWF,[46] in contrast to the high-density lipoprotein (HDL) that blocks VWF self-association.[47] In addition to its VWF clearance function, *LRP1* also inhibits inflammation – plasma concentrations of the activated complement protease C1r, inducible nitric oxide synthase, interleukin-6, and monocyte chemoattractant protein-1 (MCP-1) are

higher when LRP1 is absent.[48] Finally, *LRP1* may be associated with reduced oxidative stress.[49] Several recent studies have shown that TA-TMA pathogenesis may be related to dysregulation of the oxidative microenvironment with decreased expression of antioxidant enzymes like heme oxygenase-1[50] and nuclear factor erythroid 2-related factor 2,[51] and that its incidence may be reduced by antioxidant prophylaxis with N-acetyl cysteine.[52] Therefore, it is plausible that loss-of-function germline mutations in the *LRP1* gene predispose patients to infectious and inflammatory triggers post-transplant, which in turn decrease VWF clearance, increase inflammation/oxidative stress and complement activation, and ultimately leading to endothelial injury and TA-TMA.

The current study has several strengths. First, we carefully selected and matched the non-TMA controls on race, sex, and year of HCT, as imbalance in these traits could cause significant confounding of genetic associations. Second, we used whole exome sequencing instead of targeted NGS to examine complement and non-complement pathways simultaneously. Third, we focused on pre-specified genetic pathways based on existing biological knowledge in order to increase power and reduce multiple comparisons. Fourth, we followed a stringent variant filtering and selection process optimized for rare variant studies to reduce background signal in small sample sizes.[17] Fifth, we used modern non-burden collapsing statistical test (SKAT) to evaluate variations under unique statistical assumptions.[34]

Our study also has limitations. Despite being the largest study to date, our sample size included only 100 cases and 98 controls. However, in addition to meeting strict laboratory criteria for microangiopathic hemolytic anemia, each TA-TMA case was manually adjudicated by two reviewers to exclude known mimics such as disseminated intravascular coagulation (DIC) and disease relapse (Supplemental Table 1). The average gene sequencing depth for cases appeared slightly lower than controls so it is possible that our approach missed important variants. To mitigate this effect, we included both complement and non-complement pathways to ensure that any detected difference could be attributed to the intended genetic pathway. Third, we did not employ multiplicity adjustment due to small sample size. Therefore, any marginal p-values should be interpreted with caution. Fourth, we purposefully did not match on the acute GVHD status in the two groups because we hypothesized that GVHD may be along the causal pathway of TA-TMA. Nonetheless, the observed novel mutations among TA-TMA cases were predominantly in patients without antecedent GVHD; furthermore, our sensitivity analysis using the severe GVHD outcome did not reveal a statistical association with these genes. Finally, since we could not confirm findings from previous studies, the novel genetic associations that we report are hypothesis-generating and external validation is recommended.

In conclusion, we did not find a significant association between TA-TMA and rare variant mutations in the complement genes in adult allogeneic HCT recipients. Complement over-activation observed in TA-TMA may be an acquired phenomenon.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key points:

1. Rare variants in complement pathway genes were not associated with TA-TMA in adults.
2. Rare variants in *LRP1* in the VWF clearance pathway were associated with TA-TMA.

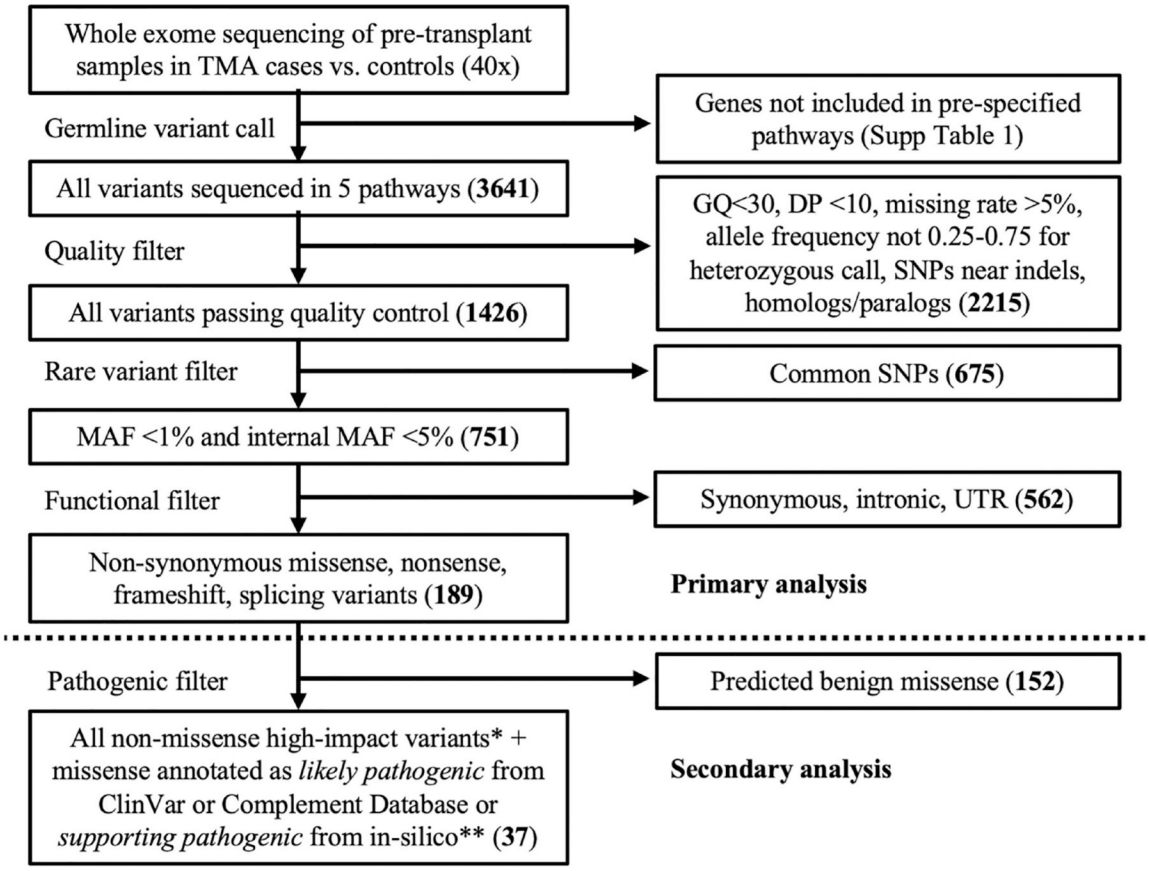

Figure 1.

GQ = genotype quality (Q30 = inferred base call accuracy 99.9%), DP = read depth at this position for this sample, missing rate = % both alleles are missing among all samples at the variant site, MAF = minor allele frequency calculated from 3 exome reference databases (GnomAD, ExAC, 100G) or if novel variant then internal MAF <5% among controls, UTR = untranslated region

*High-impact variant consequence is determined by variant effect predictor (VEP) and sequence ontology (SO) (https://useast.ensembl.org/info/genome/variation/prediction/predicted_data.html)

*In-silico prediction is determined by REVEL ensemble method (https://sites.google.com/site/revelgenomics) where a score of > 0.6 was considered “supporting pathogenic”.

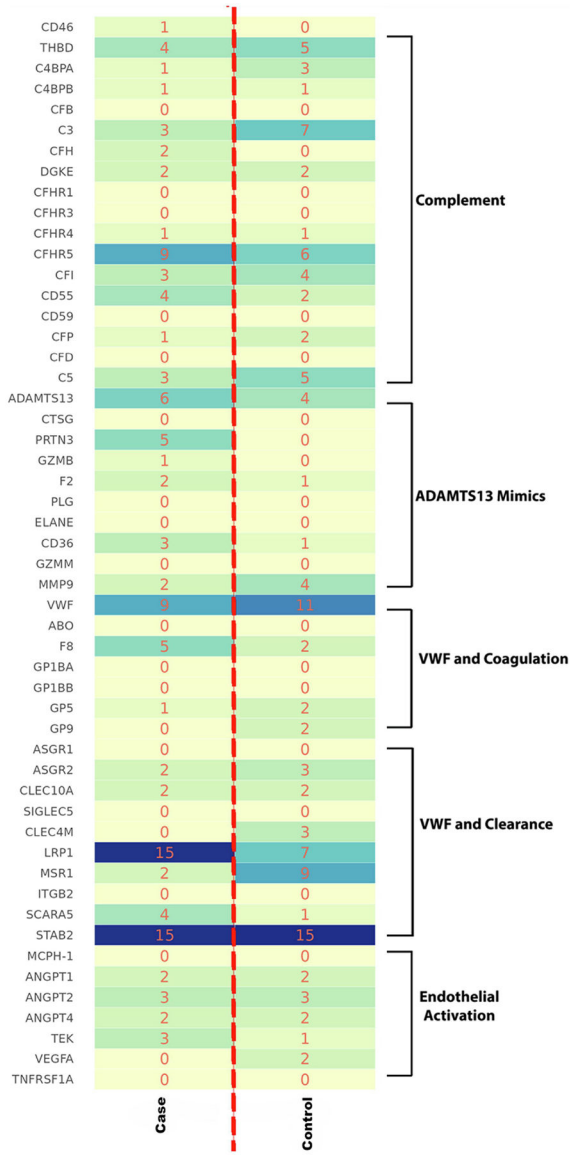

Figure 2.

Rare variants observed in samples after the functional filter are presented as the heat map. The number of variants is stratified by genes in each row and by cases and controls in each column. Different colors within each bin indicate varying ratios between the number of samples carrying variants and the number of total samples in each column. The values are increased as colors of the bins are changed from light yellow to dark blue. The numbers labeled on each bin indicate numbers of patients carrying at least 1 variant within the bin. The names of genes are labeled on the left of the heatmap, and the pathways are labeled on the right of the heatmap.

Table 1:

Patient characteristics in cases and controls

	TMA Cases (N=100)	Non-TMA Controls (N=98)	P-value
Age, median (IQR)	53 (43–60)	50 (41–61)	0.42
Female, % *	45	47	0.89
Race, % *			
White	82	83	0.62
Black	6	4	
Asian Pacific Islander	7	4	
Other or Multiple	5	8	
Body mass index, median (IQR)	28 (24–30)	27 (24–30)	0.93
Year of transplant, % *			
2006	22	18	0.98
2007	13	13	
2008	19	20	
2009	10	16	
2010	8	9	
2011	9	7	
2012	4	4	
2013	4	4	
2014	7	5	
2015	4	3	
Prior autologous transplant, % **	23	16	
Disease, %			
Myeloid malignancy	52	68	0.07
Lymphoid malignancy	40	28	
Non-malignant	8	4	
Donor matching, %			
Matched related	20	23	0.10
Matched unrelated	40	55	
Mismatched related	8	3	
Mismatched unrelated	20	12	
Umbilical cord	12	7	
Conditioning regimen, %			
Reduced intensity	41	51	0.20
Myeloablative	59	50	
GVHD prophylaxis regimen, %			
Tacrolimus-based	48	56	0.50
Cyclosporine-based	41	37	
Tacrolimus/cyclosporine + sirolimus	9	7	
Other	2	0	

	TMA Cases (N=100)	Non-TMA Controls (N=98)	P-value
GVHD development post-transplant, %			
None or grade 1	7	30	
Grade 2	44	63	<0.01
Grade 3	32	6	
Grade 4	17	1	

*Variables used in frequency matching (generates nested case-control study dataset by sampling controls from the risk set. For each case, the controls are chosen randomly from those members of the cohort who are at risk at the failure time of the case.)

**Every patient is a first allogeneic transplant recipient.

Table 2.

Pathway-specific rare variant analysis before (primary analysis) and after in-silico prediction (secondary analysis)

	Pathways	Total		TMA Cases		Non-TMA Controls		SKAT p-value
		N[1]	Variant (count)[2]	N[1]	Variant (count)[2]	N[1]	Variant (count)[2]	
Primary analysis	Complement	62	60 (75)	29	30 (36)	33	35 (39)	0.43
	ADAMTS13 and mimics	26	21 (30)	16	13 (19)	10	9 (11)	0.05
	VWF and mimics	27	31 (37)	13	16 (18)	14	17 (19)	0.47
	VWF clearance	71	58 (83)	37	35 (41)	34	30 (42)	0.07
	Endothelial activation	19	19 (20)	9	9 (10)	10	10 (10)	0.16
Secondary analysis	Complement	15	11 (15)	6	5 (6)	9	8 (9)	0.78
	ADAMTS13 and mimics	6	6 (6)	5	5 (5)	1	1 (1)	0.51
	VWF and mimics	6	6 (7)	3	3 (4)	3	3 (3)	0.50
	VWF clearance	19	11 (21)	9	7 (10)	10	5 (11)	**0.01**
	Endothelial activation	3	3 (3)	2	2 (2)	1	1 (1)	0.51

[1] Number of patients with at least 1 rare variant

[2] Number of unique rare variants (number in the parenthesis represents the total allele count among variants as multiple patients may have the same variant)

Table 3.

Gene-specific rare variant analysis before in-silico prediction

Gene	Total		TMA Cases		Non-TMA Controls		SKAT p-value
	N[1]	Variant (count)[2]	N[1]	Variant (count)[2]	N[1]	Variant (count)[2]	
PRTN3	5	1 (5)	5	1 (5)	0	0 (0)	0.01
LRP1	22	16 (22)	15	12 (15)	7	5 (7)	0.03
CLEC4M	3	1 (3)	0	0 (0)	3	1 (3)	0.04
C3	10	9 (10)	3	3 (3)	7	6 (7)	0.14
C4BPA	4	3 (4)	1	1 (1)	3	2 (3)	0.15
MMP9	6	6 (7)	2	2 (2)	4	4 (5)	0.15
ANGPT4	4	3 (4)	2	1 (2)	2	2 (2)	0.16
CD55	6	5 (6)	4	3 (4)	2	2 (2)	0.17
SCARA5	5	4 (5)	4	3 (4)	1	1 (1)	0.18
MSR1	11	7 (11)	2	2 (2)	9	6 (9)	0.19
VWF	20	20 (23)	9	10 (11)	11	11 (12)	0.27
CFHR5	15	9 (15)	9	6 (9)	6	5 (6)	0.35
F2	3	3 (3)	2	2 (2)	1	1 (1)	0.39
ANGPT2	6	6 (6)	3	3 (3)	3	3 (3)	0.40
GP9	2	2 (2)	0	0 (0)	2	2 (2)	0.40
VEGFA	2	2 (2)	0	0 (0)	2	2 (2)	0.40
C5	8	8 (8)	3	3 (3)	5	5 (5)	0.42
ASGR2	5	5 (5)	2	2 (2)	3	3 (3)	0.44
CD36	4	4 (4)	3	3 (3)	1	1 (1)	0.47
ANGPT1	4	4 (4)	2	2 (2)	2	2 (2)	0.47
CFP	3	3 (3)	1	1 (1)	2	2 (2)	0.47
F8	7	7 (9)	5	5 (6)	2	2 (3)	0.48
C4BPB	2	2 (2)	1	1 (1)	1	1 (1)	0.48
CFHR4	2	3 (3)	1	2 (2)	1	1 (1)	0.50
GZMB	1	1 (1)	1	1 (1)	0	0 (0)	0.51
TEK	4	4 (4)	3	3 (3)	1	1 (1)	0.53
CD46	1	1 (1)	1	1 (1)	0	0 (0)	0.54
CFH	2	2 (2)	2	2 (2)	0	0 (0)	0.56
STAB2	30	23 (33)	15	15 (16)	15	12 (17)	0.70
CFI	7	6 (8)	3	2 (3)	4	5 (5)	0.71
ADAMTS13	10	6 (10)	6	4 (6)	4	3 (4)	0.71
CLEC10A	4	2 (4)	2	1 (2)	2	2 (2)	0.71
THBD	9	6 (9)	4	3 (4)	5	4 (5)	0.77
GP5	3	2 (3)	1	1 (1)	2	2 (2)	0.83
DGKE	4	3 (4)	2	2 (2)	2	2 (2)	0.84

[1]Number of patients with at least 1 rare variant

[2]Number of unique rare variants (number in the parenthesis represents the total allele count among variants as multiple patients may have the same variant)

Table 4:

Gene-specific rare variant analysis after in-silico prediction

Gene	Total		TMA Cases		Non-TMA Controls		SKAT p-value
	N[1]	Variant (count)[2]	N[1]	Variant (count)[2]	N[1]	Variant (count)[2]	
LRP1	7	3 (7)	5	2 (5)	2	1 (2)	0.01
CLEC4M	3	1 (3)	0	0 (0)	3	1 (3)	0.04
MSR1	6	2 (6)	1	1 (1)	5	2 (5)	0.20
CD55	1	1 (1)	0	0 (0)	1	1 (1)	0.40
C4BPB	1	1 (1)	0	0 (0)	1	1 (1)	0.43
DGKE	1	1 (1)	0	0 (0)	1	1 (1)	0.43
VWF	4	4 (4)	1	1 (1)	3	3 (3)	0.43
CFP	1	1 (1)	0	0 (0)	1	1 (1)	0.46
SCARA5	1	1 (1)	1	1 (1)	0	0 (0)	0.48
C5	1	1 (1)	1	1 (1)	0	0 (0)	0.48
MMP9	2	2 (2)	1	1 (1)	1	1 (1)	0.48
ANGPT1	2	2 (2)	1	1 (1)	1	1 (1)	0.48
CD36	3	3 (3)	3	3 (3)	0	0 (0)	0.50
C3	1	1 (1)	1	1 (1)	0	0 (0)	0.51
STAB2	4	4 (4)	3	3 (3)	1	1 (1)	0.51
F8	2	2 (3)	2	2 (3)	0	0 (0)	0.54
TEK	1	1 (1)	1	1 (1)	0	0 (0)	0.54
F2	1	1 (1)	1	1 (1)	0	0 (0)	0.54
CFHR5	6	4 (6)	2	2 (2)	4	3 (4)	0.71
CFI	3	1 (3)	2	1 (2)	1	1 (1)	0.72

[1]Number of patients with at least 1 rare variant

[2]Number of unique rare variants (number in the parenthesis represents the total allele count among variants as multiple patients may have the same variant)