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A large-scale exome array analysis of venous thromboembolism

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No authors report a conflict of interest related to this work.

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Abstract

Although recent genome-wide association studies have identified novel associations for common variants, there has been no comprehensive exome-wide search for low-frequency variants that affect the risk of venous thromboembolism (VTE). We conducted a meta-analysis of 11 studies comprising 8,332 cases and 16,087 controls of European ancestry and 382 cases and 1,476 controls of African-American ancestry genotyped with the Illumina HumanExome BeadChip. We

used the seqMeta package in R to conduct single variant and gene-based rare variant tests. In the single variant analysis, we limited our analysis to the 64,794 variants with at least 40 minor alleles across studies (minor allele frequency ~ 0.08%). We confirmed associations with previously identified VTE loci, including *ABO, F5, F11,* and *FGA*. After adjusting for multiple testing, we observed no novel significant findings in single variant or gene-based analysis. Given our sample size, we had >80% power to detect minimum odds ratios >1.5 and 1.8 for a single variant with minor allele frequency of 0.01 and 0.005, respectively. Larger studies and sequence data may be needed to identify novel low-frequency and rare variants associated with VTE risk.

INTRODUCTION

Candidate gene studies and genome-wide association studies (GWAS) have identified multiple genetic variants that are associated with venous thromboembolism (VTE), a condition spanning both pulmonary embolism (PE) and deep vein thrombosis (DVT). The majority of genetic variants associated with VTE have been located in genes known to be involved in hemostasis, such as *ABO, F2, F5, F11, FGG and PROCR* (Crous-Bou et al., 2016). Associations have also been observed for variants located in genes outside known hemostasis pathways, such as *TSPAN15* and *SLC44A2* (Germain et al., 2015), and the exact mechanisms by which these genes influence VTE risk have yet to be determined.

Despite these successes, much of the heritability of VTE remains unexplained. A recent study based on 3,290 VTE cases and 116,868 controls from the UK Biobank estimated the heritability due to genotyped and imputed SNPs to be ~30%(Klarin et al., 2017), and twin studies have estimated VTE heritability to be as high as ~ 50%(Heit et al., 2004). However, the UK Biobank study also noted that known variants only explain 5% of VTE heritability. Thus, additional genetic variants that contribute to VTE risk remain to be discovered.

Recently developed exome arrays(Huyghe et al., 2013) allow for cost-efficient genotyping of 240,000 coding variants identified through the NHLBI Exome Sequencing Project(Tennessen et al., 2012). Based on exome and whole-genome sequencing data from 9,000 subjects of European ancestry, 2,000 subjects of African ancestry and 500 subjects each of Hispanic and Asian ancestry, 240,000 SNPs were selected for inclusion on the exome array. To be selected, non-synonymous variants had to be seen at least three times in at least two datasets whereas splice and stop variants had to be seen at least two times and in at least two datasets. The exome array has proven to be an efficient tool for identifying low-frequency coding variants associated with blood and cardiovascular traits including: hypertension(Surendran et al., 2016, Liu et al., 2016), hematological traits(Eicher et al., 2016, Chen et al., 2017),(Chami et al., 2016, Mousas et al., 2017), lipid levels(Peloso et al., 2014), coronary artery disease(Myocardial Infarction et al., 2016), and atrial fibrillation(Christophersen et al., 2017). However, no study has published a comprehensive investigation of the association between low-frequency exonic variants and VTE.

We hypothesized that exonic, low-frequency genetic variation would be associated with VTE. We meta-analyzed exome array genotype data from 11 European and US studies, totaling 8,723 VTE cases and 17,563 controls. We conducted both single-variant and gene-based tests to identify novel genetic variants associated with VTE risk.

MATERIALS AND METHODS

Study participants

All study participants were either of European or African-American ancestry and came from eight US-based cohorts (ARIC, CHS, FHS, HPFS, NHS, NHSII, WGHS and WHI), one US-based case-control study (HVH), one Norwegian case-control study (Tromsø) and one French case-control study (MARTHA)(ARIC Investigators, 1989, Fried et al., 1991, Tell et al., 1993, Germain et al., 2011, Hankinson et al., 1995, Tworoger et al., 2006, Ridker et al., 2008, Solomon et al., 2016, Kannel et al., 1961, Giovannucci et al., 1995, Heckbert et al., 2008, Women's Health Initiative Study Group, 1998, Psaty et al., 2009) (Table 1). Details of each study have been previously published(ARIC Investigators, 1989, Fried et al., 1991, Tell et al., 1993, Germain et al., 2011, Hankinson et al., 1995, Tworoger et al., 2006, Ridker et al., 2008, Solomon et al., 2016, Kannel et al., 1961, Giovannucci et al., 1995, Heckbert et al., 2008, Women's Health Initiative Study Group, 1998). Physician-diagnosed VTE was identified either through hospital records or validated self-reports, supplemented by review of medical records. A detailed description of the study-specific design and characteristics is presented in Supplementary Table 1. All participating studies were approved by their respective institutional review board and informed consent for genetic analyses was obtained from each study participant.

Genotyping and quality control

Genotyping was conducted using either the Illumina HumanExome BeadChip v1.0, Illumina HumanExome BeadChip v1.1 or the Illumina HumanCore Exome BeadChip v1.1, depending on study. Genotypes from 765 samples from the Tromsø study were obtained from exome sequencing rather than genotyping. Genotypes were called using either GenomeStudio or Zcall. Each study conducted data cleaning and quality assurance checks following a common protocol. Details of study-specific genotype calling and quality control can be found in Supplementary Table 1.

We conducted gene set enrichment analysis using the GSEAPreranked algorithm as implemented in the GenePattern software and the KEGG, Gene Ontology, and Hallmarks pathway sets(Mootha et al., 2003, Subramanian et al., 2005). We applied GSEAPreranked to four sets of results (1) Burden test of variants using a broad definition, (2) Burden test of variants using a strict definition, (3) SKAT test of variants using a broad definition, (4) SKAT test of variants using a strict definition.

One of the included studies (MARTHA) genotyped cases and controls on separate platforms (cases were genotyped on Illumina HumanExome 12v.1–2_A and controls were genotyped with the Illumina HumanExome 12v.1_A array). We identified a moderate inflation in test statistics (λ_{1000}=1.09) in MARTHA for the single variant analysis with MAF>0.005. Therefore, we re-meta-analyzed the data while excluding MARTHA, leaving a total of 6,095 cases and 14,149 controls in a sensitivity analysis.

Statistical Methods

Each study conducted individual analysis following a common protocol. To avoid type-1 error inflation(Ma et al., 2013), studies with more than four controls per case randomly selected a maximum of four controls for each case (i.e. 1:4 case:control ratio). Those studies that performed control selection (ARIC, CHS, FHS, WGHS and WHI) reviewed the distribution of age and sex following control selection to ensure roughly equal distributions among cases and controls. Each study conducted both single variant and gene-based analysis. Association analysis were based on logistic regression adjusting for age, sex, principal components and other study-specific variables (as needed). Analyses were conducted using the seqMeta(Voorman A, 2017) package in R which produces study-specific results. Each study sent their study-specific results to the coordinating center at Harvard T.H. Chan School of Public Health where the meta-analyses took place.

For single variant analysis, we conducted a meta-analysis of the study-specific score statistics based on an additive coding. We limited our analysis to the 64,794 variants that had at least 40 minor alleles across studies (corresponding to a minor allele frequency of ~ 0.08%). Bonferroni correction for the number of variants tested was used to set the significance threshold for the analysis corresponding to $P<7.7\times10^{-7}$ (0.05/64,794 variants).

For the gene-based rare variant analyses, we conducted two tests: Weighted-Sum Burden(Madsen and Browning, 2009) (WSB) test as implemented in seqMeta(Voorman A, 2017) and SKAT(Wu et al., 2011). We applied two different sets of criteria to select variants, based on coding variant annotation from five prediction algorithms (PolyPhen2, HumDiv and HumVar, LRT, MutationTaster and SIFT)(Purcell et al., 2014). The ‘broad’ definition included variants with a minor allele frequency (MAF) < 0.01 that were nonsense, stop-loss, splice site, as well as missense variants that are annotated as damaging by at least one prediction algorithm. The ‘strict’ definition included only variants with a MAF < 0.01 that were nonsense, stop-loss, splice-site, as well as missense variants annotated as damaging by all five algorithms. For the SKAT analysis, variants were weighted according to the beta density function as previously described(Wu et al., 2011). We excluded all genes that had fewer than two variants included in the analysis. In total, we tested 15,041 genes using the broad definition and 5,749 genes using the strict definition. Bonferroni correction for the number of genes and tests performed was used to set the significance threshold for the gene-based analysis corresponding to $P<1.2\times10^{-6}$ (0.05/41,580 tests [(5,749+15,041) genes × 2 tests]).

One of the included studies (MARTHA) genotyped cases and controls on separate platforms (cases were genotyped on Illumina HumanExome 12v.1–2_A and controls were genotyped with the Illumina HumanExome 12v.1_A array. We identified a moderate inflation in test statistics (λ_{1000}=1.09) in MARTHA for the single variant analysis with MAF>0.005. Therefore, we reran the analysis excluding MARTHA, leaving a total of 6,095 cases and 14,149 controls in a sensitivity analysis.

RESULTS

Single Variant Analysis

After excluding all variants with MAC<40 (corresponding to a minor allele frequency of ~ 0.08%) in the combined study population, single variant meta-analysis showed no sign of genomic inflation (λ_{1000}=1.03, Supplementary Figure 1). The strongest association was observed for rs635634 at the *ABO* locus (OR=1.60, 95% CI: 1.52–1.68, P=1.51×10^{-73}). In addition, we observed significant associations for previously known genes including the *F5, FGG, and F11* (Supplementary Table 2). The most strongly associated rare (MAF<0.01) variant we observed was rs121918472 (OR=1.93, 95% CI: 1.46–2.56, P=3.55×10^{-6}), a non-synonymous variant located in the Protein S (*PROS1*) gene, also known as the p.Ser501Pro or PS Herleen mutation, MAF=0.005. This variant is also known to be associated with VTE(Suchon et al., 2017). After excluding known loci, only one single variant remained significant after adjusting for multiple testing but this signal was driven exclusively by the MARTHA study (p=1.96×10^{-15} when including MARTHA, p=0.37 after excluding MARTHA). As the signal at this locus is most likely due to technical issues, we removed it from further analysis. No other variant reached genome-wide significance (P<7.7×10^{-7}). The strongest sub-threshold association was observed for rs755109, a common (MAF=0.37) variant previously associated with thyroid stimulating hormones (OR=1.10, 95% CI: 1.06–1.16, P=3.31×10^{-6}).

Weighted-Sum Burden (WSB) Rare Variant Analysis

No gene reached the pre-determined significance threshold of P<1.2×10^{-6} (Figure 1). The top three associated genes using the ‘broad’ and ‘strict’ definitions are shown in Table 2 and all associations with p<0.01 are shown in Supplementary Tables 3 & 4. The *SERPINA10* gene on chromosome 14 was the third strongest associated gene using the broad (p=0.0002) and the strict (p=0.0007) definition. *SERPINA10* is expressed primarily in the liver and mutations in this gene have previously been linked to VTE(Van de Water et al., 2004, Corral et al., 2006).

SKAT Rare Variant Analysis

No gene reached the pre-determined significance threshold of P<1.2×10^{-6} (Figure 1). The top three associated genes using the using the ‘broad’ and ‘strict’ definitions are shown in Table 2 and all associations with p<0.01 are shown in Supplementary Tables 5 & 6.

Gene Set Enrichment Analysis

Gene set enrichment analysis based on the results obtained from WSB and SKAT did not yield any significant pathway after adjusting for number of pathways tested (all FDR q>0.08, data not shown).

DISCUSSION

To assess the contribution of rare coding variation to VTE risk, we combined data from 11 studies spanning four countries, resulting in exome array data on 8,723 VTE cases and 17,563 controls. By comparison, the number of cases included in this study is larger than the

largest GWAS of VTE published to date(Germain et al., 2015). Beyond known associations, we did not observe evidence that additional low-frequency and rare coding variants with moderate-to-large effects contribute to VTE risk.

Although our study is the largest genomic study of VTE to date, our ability to identify rare variants associated with VTE was limited by low statistical power. Given our sample size, we had >80% power to detect minimum odds ratios of 1.56 and 1.81 for a single variant with MAF = 0.01 and 0.005, respectively. It is estimated that the exome array includes 97–98% of non-synonymous variants and 94–95% of stop variants that would have been detected in an average genome through exome sequencing (https://genome.sph.umich.edu/wiki/Exome_Chip_Design). Thus, it is possible that we missed coding variants associated with VTE, especially if such variants are particularly rare in individuals who are not affected with VTE. Another limitation with this study is the limited contribution of non-European populations to our analyses, with 93% of our study population being of European ancestry.

Identifying risk factors for VTE, including genetic risk factors, is of great public health importance. VTE affects 1–2/1000 Americans yearly. The incidence has been increasing and mortality from PE remains high(Arshad et al., 2017, Heit et al., 2017, Silverstein et al., 1998, Heit, 2005). The mortality of VTE is greatest in the first 24 hours, and for one-fourth of PE patients, the initial clinical presentation is sudden death(Goldhaber et al., 1999, Courtney and Kline, 2001, Cohen et al., 2007). Therefore, our ability to improve mortality hinges on primary prevention, identifying patients at risk for VTE, and understanding the underlying pathophysiology of the disease. Despite the accumulated evidence that genetic factors play a major role in the pathophysiology of VTE, only 35% of VTE patients undergoing testing for thrombophilia carry a polymorphism known to increase VTE risk(Cushman, 2005). Additional efforts to identify genetic variants associated with VTE risk are still needed.

The INVENT Consortium is a well-established collaboration of genetic studies of VTE and, to our knowledge, our meta-analysis includes data from the vast majority of exome array studies of VTE. Additional large studies, potentially including comprehensive sequencing data, may be needed to identify novel low-frequency rare coding variants associated with VTE. Further research into the genetic basis of VTE is needed to aid in the primary prevention of this potentially fatal disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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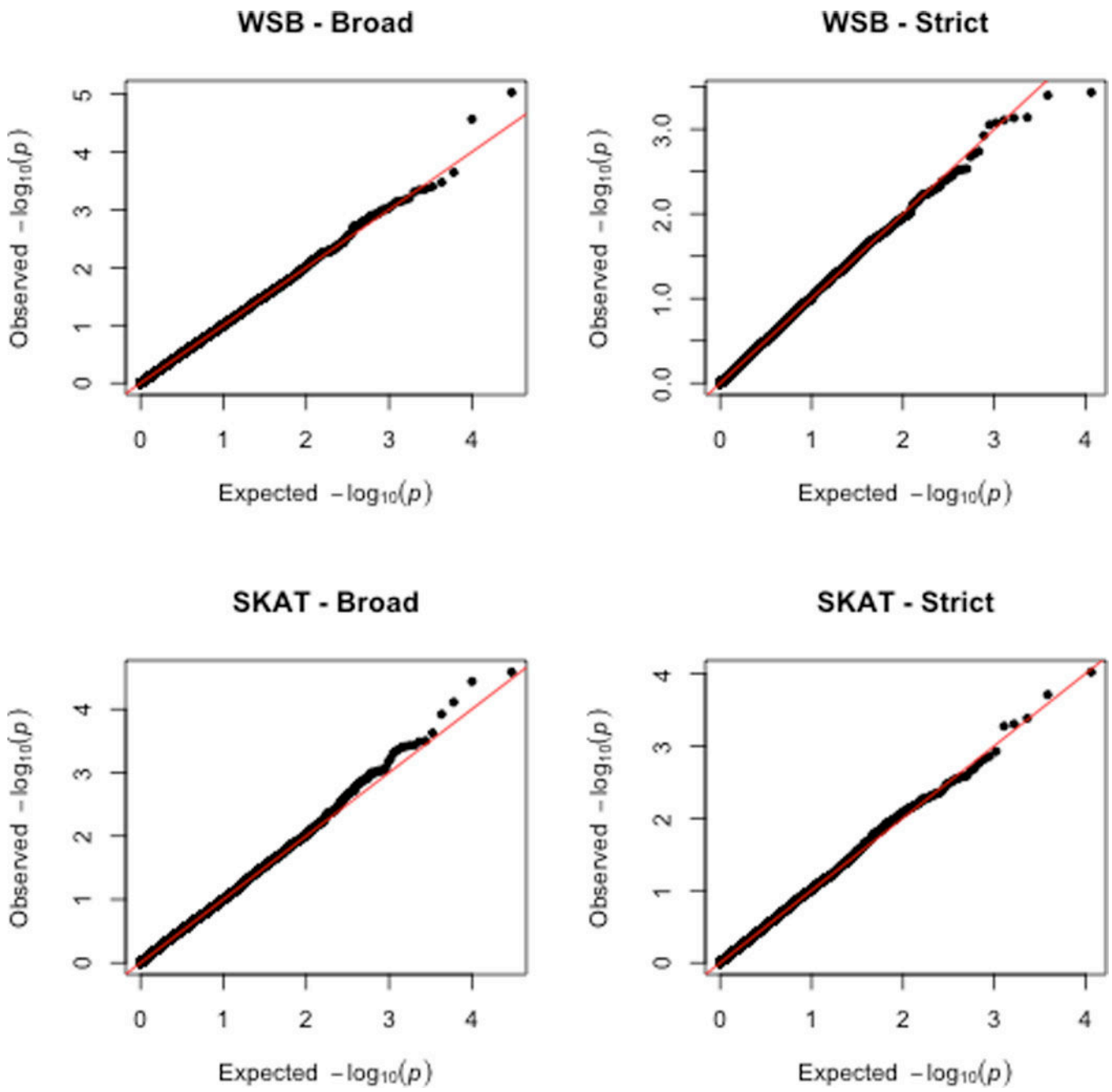

Figure 1:
QQ plots for gene burden tests including non-synonymous variants with MAF≤0.01. The WSB test using a broad definition of variant inclusion (upper left panel), the WSB test using a strict definition of variant inclusion (upper right panel), the SKAT test using a broad definition of variant inclusion (lower left panel), the SKAT test using a strict definition of variant inclusion (lower right panel).

Table 1:

Studies included in the VTE exome array analysis.

Ethnicity	Study	Country	Cases	Controls
African-American	ARIC	US	202	807
African-American	CHS	US	30	120
African-American	HVH	US	58	181
African-American	WHI	US	92	368
European	ARIC	US	433	1,734
European	CHS	US	112	448
European	FHS	US	212	848
European	HPFS/NHS/NHSII	US	2,321	2,301
European	HVH	US	841	1,788
European	MARTHA	France	2,628	3,414
European	Tromsø	Norway	528	526
European	WGHS	US	610	2,404
European	WHI	US	656	2,624
	Total		**8,341**	**16,087**

Table 2:

Association results for the three strongest associations from the rare variant WSB test.

Variant Inclusion	Gene	Beta	SE	*P*	CMAF*	# Variants†
Broad	*FAM71C*	3.86	0.871	9.30E-06	0.0002	2
	FOXB2	1.56	0.372	2.71E-05	0.0009	4
	SERPINA10	0.28	0.077	0.0002	0.017	8
Strict	*DGAT2*	0.64	0.179	0.0004	0.003	4
	NUDT12	3.11	0.877	0.0004	0.0002	2
	SERPINA10	0.28	0.082	0.0007	0.015	6

*CMAF = Cumulative MAF for SNPs included in the analysis

†Number of variants included in the analysis

Table 3:

Association results for the three strongest associations from the rare variant SKAT test.

Variant Inclusion	Gene	Qmeta*	*P*	CMAF†	# Variants‡
Broad	*CREB3L1*	596343.13	2.59E-05	0.016	7
	FAM71C	9844.60	3.65E-05	0.0002	2
	PHC3	514613.94	7.76E-05	0.017	10
Strict	*SRR*	37032.58	9.52E-05	0.0007	4
	ABCF3	15590.02	0.0002	0.0007	2
	DSC1	50186.59	0.0004	0.0014	5

*The SKAT Q statistic, defined as $\sum_j w_j U_j^2$, where wj is the weight given to SNP j, and U_j^2 is the associated score statistic

†CMAF = Cumulative MAF for SNPs included in the analysis

‡Number of Variants included in the analysis