

Genetic Variants in *LRP1* and *ULK4* Are Associated with Acute Aortic Dissections

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Acute aortic dissections are a preventable cause of sudden death if individuals at risk are identified and surgically repaired in a non-emergency setting. Although mutations in single genes can be used to identify at-risk individuals, the majority of dissection case subjects do not have evidence of a single gene disorder, but rather have the other major risk factor for dissections, hypertension. Initial genome-wide association studies (GWASs) identified SNPs at the *FBN1* locus associated with both thoracic aortic aneurysms and dissections. Here, we used the Illumina HumanExome array to genotype 753 individuals of European descent presenting specifically with non-familial, sporadic thoracic aortic dissection (STAD) and compared them to the genotypes of 2,259 control subjects from the Atherosclerosis Risk in Communities (ARIC) study matched for age, gender, and, for the majority of cases, hypertension. SNPs in *FBN1*, *LRP1*, and *ULK4* were identified to be significantly associated with STAD, and these results were replicated in two independent cohorts. Combining the data from all cohorts confirmed an inverse association between *LRP1* rs11172113 and STAD ($p = 2.74 \times 10^{-8}$; OR = 0.82, 95% CI = 0.76–0.89) and a direct association between *ULK4* rs2272007 and STAD ($p = 1.15 \times 10^{-9}$; OR = 1.35, 95% CI = 1.23–1.49). Genomic copy-number variation analysis independently confirmed that *ULK4* deletions were significantly associated with development of thoracic aortic disease. These results indicate that genetic variations in *LRP1* and *ULK4* contribute to risk for presenting with an acute aortic dissection.

The major diseases affecting the thoracic aorta are aortic aneurysms and acute aortic dissections. The natural history of ascending thoracic aortic aneurysms is to asymptotically enlarge over time until an acute tear in the intimal layer leads to an ascending aortic dissection (termed Stanford type A dissections). Type A aortic dissections cause sudden death in up to 40% of individuals and are associated with a high degree of morbidity and medical expenditure in survivors. Less deadly aortic dissections originate in the descending thoracic aorta just distal to the branching of the subclavian artery (type B dissections) and are part of the spectrum of disease. Prevention of premature death from aortic dissections depends on the early identification of individuals at risk, careful monitoring of the aorta for aneurysms, medications to slow the rate of growth of aneurysms, and timely surgical repair of aneurysms.¹

Family aggregation studies indicate more than 20% of individuals with thoracic aortic aneurysms and dissections (TAAD) have a family history of disease that can be due to a genetic syndrome resulting from a single gene mutation such as Marfan syndrome (MFS [MIM: 154700]) due to *FBN1* (fibrillin-1 [MIM: 134797]) mutations or an autosomal-dominant condition in the absence of syndromic

features, termed familial TAAD (FTAAD).^{2,3} Mutations in genes that encode proteins involved in smooth muscle cell (SMC) contraction and adhesion to the extracellular matrix or the TGF- β signaling pathway have been identified to be a cause of heritable thoracic aortic disease, including *MYH11* (smooth muscle myosin heavy chain [MIM: 160745]), *ACTA2* (smooth muscle α actin [MIM: 102620]), *MYLK* (myosin light chain kinase [MIM: 600922]), *PRKG1* (cGMP-dependent protein kinase type I [MIM: 76894]), *FBN1*, *TGFBR1* (transforming growth factor β receptor 1 [MIM: 190181]), *TGFBR2* (transforming growth factor β receptor II [MIM: 190182]), *TGFBR2* (transforming growth factor β 2 [MIM: 190220]), and *SMAD3* (SMAD family member 3 [MIM: 603109]).^{4–11} Additional genes with mutations that cause thoracic aortic disease have been identified more recently and encode proteins involved in the structure of the extracellular matrix, *MFAP5* (microfibrillar-associated protein 5 [MIM: 601103]) and *LOX* (lysyl oxidase [MIM: 153455]), or proteins involved in SMC metabolism or survival, *MAT2A* (methionine adenosyltransferase ii, alpha [MIM: 601468]) and *FOXEO3* (forkhead box e3 [MIM: 601094]).^{12–15} In TAAD-affected case subjects without a

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Table 1. Clinical Characteristics of the Discovery and Replication Cohorts

Variable ^a	Discovery Cohort Controls (n = 2,259)	Discovery Cohort Cases (n = 753)	Replication Cohort 1 (n = 108)	p Value ^b	Replication Cohort 2 (n = 114)
Age (years)	62 (58–63)	61.0 (53.0–69.8)	58 (48–67)	0.002	64.0 (56.0–75.5)
Male	1,719 (76.1)	272 (76)	80 (74.1)	0.669	90 (73.2)
Hypertension	1,506 (66.7)	643 (84.5)	89 (82.4)	0.219	107 (88.4)
Smoking (past or present)	1,477 (65.4)	416 (55.2)	69 (63.8)	0.367	65 (57.0)
Type A dissection	0	469 (62.3)	62 (57.4)	0.330	75 (61.0)
Type B dissection	0	288 (38.2)	48 (44.4)	0.217	48 (39.0)
Bicuspid aortic valve	0	55 (7.3)	8 (7.4)	0.681	9 (7.3)

^aAge is summarized as median (interquartile range); other variables are presented as frequency, n (%).

^bChi square test for discovery cohort and replication cohort 1. Individual data for replication cohort 2 was unavailable.

known single gene disorder, the major risk factor is hypertension.¹⁶

To identify genetic factors that predispose individuals to non-familial thoracic aortic disease, we previously pursued a GWAS with 765 European American (EA) case subjects presenting with either thoracic aortic aneurysms or aortic dissections. SNPs spanning *FBNI* at 15q21.1 locus were significantly associated with an increased risk of developing sporadic TAAD (STAAD), including type A sporadic thoracic aortic dissection.¹⁷ The SNP array data was also used to identify recurrent chromosome 16p13.1 duplications involving *MYH11*, which were significantly associated with an increased risk of both type A and B aortic dissections.¹⁸

To further pursue genetic variants for thoracic aortic disease, we focused on case subjects with acute aortic dissections, the life-threatening presentation of thoracic aortic disease. Individuals with dissections with syndromic features of MFS or Loeys-Dietz syndrome (MIM: 610168), a family history of TAAD, or who were under the age of 30 years were excluded from this study. Blood or saliva samples and clinical information were collected from EA and European case subjects presenting with ascending or descending (Stanford type A or B) sporadic thoracic aortic dissections (STADs) after obtaining approval from the Institutional Review Board of the University of Texas Health Science Center at Houston, Baylor College of Medicine, the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC) cohort, University of Michigan, and Karolinska University Hospital Solna, Sweden, and proper informed consent was obtained from the participants at each institution. For the discovery study, we genotyped 780 DNA samples using the Illumina HumanExome v.1.0 array. Quality-control procedures excluded samples with >3% of genotypes missing, population clustering outliers via principal-components analysis (± 6 SD from the mean), individuals with high inbreeding coefficients or heterozygote rates, individuals with gender mismatches, one individual from a duplicate pair, and samples with a high degree of relatedness (π hat > 0.9). Variants with a call

rate less than 0.97 and autosomal variants with minor allele frequency (MAF) ≥ 0.05 and Hardy Weinberg equilibrium p value $< 1 \times 10^{-6}$ were also removed. The 753 case subjects used for subsequent analyses were predominantly men (76%) with an average age at presentation of dissection of 61 years, 84% were hypertensive, 55% had a history of smoking, and 7% had bicuspid aortic valve (BAV) (Table 1). This cohort included 462 (62%) case subjects with type A dissection and 291 (38%) case subjects with type B dissection.

For the case-control association study, we obtained genotype data using the Illumina HumanExome v.1.0 array on 2,259 individuals from the Atherosclerosis Risk in Communities (ARIC) study as control subjects matched by descending age rank, gender, and ethnicity. Because of limited data from control subjects, two-thirds of the control subjects were matched for hypertension and one-third were nonhypertensive. ARIC participants who had TAAD, myocardial infarction, or stroke before age of 65 years old were excluded as control subjects. Quality control analyses of ARIC exome chip data were previously described.^{19,20} To test association of variants with STAD, SNP-based case-control association studies were performed on the variants with MAF equal to or greater than 0.05 using Firth logistic regression models.²¹ Comparison of the distribution of observed to expected p values is shown in a quantile-quantile (Q-Q) plot (Figure S1). Variants on the exome chip were annotated using dbNSFP v.2.0.²²

SNPs in *FBNI*, *LRP1* (low-density lipoprotein receptor-related protein 1 [MIM: 107770]), and *ULK4* (unc-51 like kinase 4) were identified to be associated with STAD with an exome-wide significance level ($p < 0.05$ divided by the number of variants tested, i.e., $p < 2.0 \times 10^{-6}$; Table 2). Association of SNP rs1042078 at 3' UTR of *FBNI* with STAD ($p = 1.9 \times 10^{-10}$) confirmed our previous GWAS finding that *FBNI* SNPs in a haplotype block were significantly associated with an increased risk of thoracic aortic dissection.¹⁷ An intronic SNP in *LRP1* (rs11172113 on chromosome 12, MAF = 0.38 in the ARIC control subjects) was identified to be significantly associated with a decreased risk for STAD ($p = 4.2 \times 10^{-7}$). Based on HapMap

Table 2. Association of SNPs in *FBN1*, *LRP1*, and *ULK4* with STAD and Type A Dissection

Disease	Gene	SNP	Discovery Cohort		Replication Cohort 1		Replication Cohort 2		Combined Analysis	
			p ^a	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)
STAD-Type A+B	<i>FBN1</i>	rs1042078	1.08 × 10 ⁻¹⁰	1.51 (1.33–1.70)						
	<i>LRP1</i>	rs11172113	4.21 × 10 ⁻⁷	0.73 (0.65–0.83)	0.11	0.75 (0.53–1.06)	0.02	0.73 (0.56–0.95)	2.74 × 10 ⁻⁸	0.82 (0.76–0.89)
	<i>ULK4</i>	rs2272007	1.34 × 10 ⁻⁶	1.44 (1.24–1.66)	0.01	1.67 (1.12–2.49)	0.02	1.44 (1.07–1.94)	1.15 × 10 ⁻⁹	1.35 (1.23–1.49)
STAD-Type A	<i>FBN1</i>	rs1042078	8.46 × 10 ⁻⁹	1.55 (1.33–1.79)						
	<i>LRP1</i>	rs11172113	2.26 × 10 ⁻⁴	0.73 (0.63–0.85)	0.11	0.71 (0.46–1.08)	0.03	0.67 (0.47–0.97)	3.11 × 10 ⁻⁶	0.81 (0.75–0.89)
	<i>ULK4</i>	rs2272007	6.58 × 10 ⁻⁶	1.49 (1.26–1.77)	0.04	1.66 (1.03–2.68)	0.05	1.45 (0.99–2.13)	3.55 × 10 ⁻⁸	1.39 (1.23–1.56)

^aSignificant p values were defined as $p < 0.05$ divided by the number of variants tested, which was 1.96×10^{-6} for discovery phase in this study.

data, rs11172113 is located between two haplotype blocks (Figure 1A). A nonsynonymous SNP in *ULK4* (rs2272007 on chromosome 3, MAF = 0.18 in the ARIC control subjects) showed significant association with STAD ($p = 1.2 \times 10^{-6}$) and another eight SNPs in *ULK4* were associated with STAD but did not reach genome-wide significance (p values between 8.5×10^{-5} and 3.2×10^{-6}) (Table S1). Based on HapMap CEU data v.4.2, all nine *ULK4* SNPs are located within a 291 kb haplotype block between SNPs rs9847006 and rs9856633, and rs2272007 is a tag SNP for this block (Figure 1B). Thus, the minor alleles of the nine *ULK4* SNPs are located at the same haplotype block, and we selected rs2272007 to test in the replication studies.

To validate these findings, we obtained genotype data for *LRP1* rs11172113 and *ULK4* rs2272007 from two replication cohorts. The first replication cohort included 129 EA case subjects with STAD and 181 ethnicity-matched control subjects. The second replication cohort included 123 EA STAD case subjects from the Cardiovascular Health Improvement Project (CHIP) with STAD and 13,646 ethnicity-matched control subjects from the Michigan Genomics Initiative (MGI) study. *LRP1* rs11172113 and *ULK4* rs2272007 were significantly associated with an increased risk of STAD in these cohorts (Table 2). Combining the data from all cohorts (985 case subjects with STAD, including 606 case subjects with type A dissection and 16,086 control subjects) resulted in a highly significant association of *LRP1* rs11172113 with a decreased risk for STAD and type A dissection alone with p values of 2.7×10^{-8} (OR = 0.82, 95% CI = 0.76–0.89) and 3.1×10^{-6} (OR = 0.81, 95% CI = 0.75–0.89), respectively, and *ULK4* rs2272007 with an increased risk for STAD and type A dissection with p values of 1.2×10^{-9} (OR = 1.35, 95% CI = 1.23–1.49) and 3.6×10^{-8} (OR = 1.39, 95% CI = 1.23–1.59), respectively. Previous studies found that the same *ULK4* SNPs associated with STAD are also associated with hypertension.¹⁶ The majority of the control subjects in the discovery stage of the study were matched for hypertension, suggesting that these *ULK4* SNPs predispose to

aortic dissection independent of the risk for hypertension. It is also notable that no other loci associated with hypertension were found to also be associated with STAD in this study.^{23–25}

To further investigate whether *ULK4* variants predispose to thoracic aortic disease, we investigated whether copy-number variants (CNVs) in *ULK4* were present in individuals with sporadic TAAD (STAAD). CNV analysis of the *ULK4* locus was done using SNP array data on 1,390 case subjects, including 801 case subjects of EA descent with STAAD,¹⁷ 109 individuals with early-onset TAAD (ETAAD) who were diagnosed with TAAD before 30 years old, and 480 subjects with STAAD with bicuspid aortic valve who underwent surgical repair at Harvard Medical School (BWH). CNVs were analyzed using PennCNV and CMVPartition programs and methods described previously.^{18,26} Three *ULK4* genomic deletions, which were between 119 and 464 kb pairs in length and predicted to remove 2 to 16 exons of *ULK4*, were identified (Figure 2 and Table 3). One of these *ULK4* deletions led to haploinsufficiency (frameshift mutation leading to nonsense-mediated decay), and two are in-frame deletions of either 124 or 576 amino acids. We detected one deletion involving *ULK4* in 15,446 qualified control subjects from the Database of Genotypes and Phenotypes (dbGAP), including studies of Genetic Epidemiology of Refractive Error in the KORA Study ($n = 1,865$; dbGAP: phs000303.v1.p1), the Health and Retirement Study ($n = 9,428$; dbGAP: phs000428.v1.p1), a GWA study of Fuchs' Endothelial Corneal Dystrophy ($n = 3,218$; dbGAP: phs000421.v1.p1), and the Genetic Architecture of Smoking and Smoking Cessation study ($n = 935$; dbGAP: phs000404.v1.p1). We did not detect any duplications involving *ULK4* in case or control subjects. Therefore, deletions in *ULK4* increase the odds ratio for the association of *ULK4* deletions and TAAD (OR = 16.7, 95% CI 1.7–160; $p = 0.0009$). The association of *ULK4* deletions with TAAD independently provides further support that genetic variations in *ULK4* contribute to the pathogenesis of TAAD. Because there is a low frequency of *ULK4* in individuals with thoracic aortic disease, future

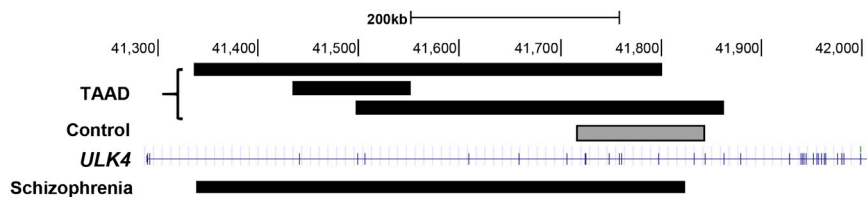


Figure 2. Characterization of *ULK4* Deletions in Individuals with Thoracic Aortic Disease and a Control Subject

The black bars indicate the extent of genomic deletion in individuals with TAAD and the gray bar indicates the extent of genomic deletion identified in a control subject. The bar below the schematic of the *ULK4* gene structure indicates the extent of the typical *ULK4* deletion identified in individuals with schizophrenia. The scale is in kilobases.

CNV studies need to be conducted to confirm the association of *ULK4* deletion with STAD. Recurrent genomic deletions of *ULK4* have been reported in individuals with schizophrenia and were suggested to be a rare susceptibility gene for schizophrenia.²⁷ Note that the STAD-affected case subjects who have *ULK4* deletions do not have schizophrenia.

LRP1 encodes low-density lipoprotein receptor-related protein 1, which is highly expressed in vascular SMCs, neurons, macrophages, and fibroblasts. Mice with SMC-specific knockout of *Lrp1* have aortic disease including tortuous aortas, elastic lamellae disruption, and evidence of increased TGF- β signaling.²⁸ *LRP1* rs11172113, which is associated with a decreased risk of STAD, is located in between two haplotype blocks (Figure 1A). Interestingly, *LRP1* rs11172113 has been reported to be associated with migraines in multiple populations and ischemic stroke in an African American cohort.^{23,29} Consistent with our study, the rs11172113 minor allele was associated with a decreased risk of developing vascular diseases in these studies. In contrast, another *LRP1* SNP, rs1466535, which is located in an adjacent haplotype block to rs11172113, is significantly associated with increased risk for abdominal aortic aneurysm²⁴ and is found not to be associated with carotid artery disease.²⁵ Based on our data and these reports, different *LRP1* variants can either protect or increase the risk for specific vascular diseases.

This study identified *ULK4* SNP rs2272007 as significantly associated with STAD, along with marginally significant association of eight other SNPs in the same haplotype block (Figure 1B). The increased burden of *ULK4* genomic deletions in TAAD-affected case subjects provided additional evidence that variants in *ULK4* contribute to an

increased risk for thoracic aortic disease. *ULK4* encodes unc-51-like kinase 4 and the majority of the mice with knockout of *Ulk4* died before reaching 4 months of age. The *Ulk4*^{-/-} mice have congenital hydrocephalus and marked to severe dilatation of the lateral and third ventricles.³⁰ Multiple GWASs have identified significant association of the same *ULK4* SNPs with hypertension in individuals of EA, African American, and East Asian descent, and diastolic blood pressure in EA.³¹⁻³³ Long standing and poorly controlled hypertension is a major risk factor for STAAD. Therefore, to avoid hypertension as a confounder for the association study, the majority of the ARIC control subjects used for the discovery study were matched for hypertension. To further investigate whether association of *ULK4* SNP, rs2272007 with STAD was due to the association with hypertension, we tested the association of rs2272007 with hypertension in STAD-affected case subjects or case subjects with type A dissection in the discovery cohort study. We also tested whether SNPs in the other genes associated with hypertension were associated with STAD or type A dissection. No significant association was observed in SNPs in genes that were reported to be associated with hypertension or blood pressure, including *ADRB1* (MIM: 109630), *AGT* (MIM: 106150), *ATP2B1* (MIM: 108731), *CACNB2* (MIM: 600002), *CASZ1* (MIM: 609895), *CYP17A1* (MIM: 609300), *FGF5* (MIM: 165190), *GUCY1A3* (MIM: 139396), *HFE* (MIM: 613609), *MTHFR* (MIM: 607093), *NPPA* (MIM: 108780), *NPPB* (MIM: 600295), *NPR3* (MIM: 108962), *PLEKHG1*, *SLC4A7* (MIM: 603353), *SOX6* (MIM: 607257), *TBX3* (MIM: 601621), and *TBX5* (MIM: 601620). These results suggest that in addition to the association with hypertension, genomic variants in *ULK4* have a different mechanism for contributing to the pathogenesis of STAD.³¹⁻³⁴

Table 3. TAAD-Affected Case Subjects with *ULK4* Deletions

Case	Deletion (GRCh37)	Patient Cohort	Ethnicity	Gender	Age onset of TAAD	Aneurysm	Dissection	Hypertension	BAV	Schizophrenia	cDNA Deleted (bp)
1	chr3: 41,387,911-41,851,718	STAAD	white	M	53	yes	no	no	no	no	1,483
2	chr3: 41,432,658-41,551,187	STAAD	white	M	67	yes	no	yes	no	no	372
4	chr3: 41,494,618-41,859,973	ETAAD	white	M	59	yes	no	NA	yes	no	1,728

In summary, we identified that SNPs in two genes, *LRP1* and *ULK4*, were significantly associated with acute aortic dissections in patients who did not have syndromic features or a family history of the disease. Interestingly, association of *ULK4* deletions with TAAD was found, further confirming that *ULK4* genetic variants are involved in the pathogenesis of STAD. Common variants in *LRP1* have been found to be associated with a variety of vascular diseases, and the inverse association of a *LRP1* SNP with aortic dissections further confirms a role of variants in this gene contributing to vascular diseases. Our data support that the genetic variants in *ULK4* predispose individuals to STAD independent of the increased risk for hypertension.

Accession Numbers

The accession number of the deletion involving *ULK4* is dbVar: nstd129.

Supplemental Data

Supplemental Data include consortia members, one figure, and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2016.06.034>.

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Web Resources

CADD, <http://cadd.gs.washington.edu/>
dbGaP, <http://www.ncbi.nlm.nih.gov/gap>
dbNSFP v.2.0, <https://sites.google.com/site/jpopgen/dbNSFP>
dbSNP, <http://www.ncbi.nlm.nih.gov/projects/SNP/>
dbVar, <http://www.ncbi.nlm.nih.gov/dbvar/>
Ensembl Genome Browser, <http://www.ensembl.org/index.html>
ExomeChip – Charge Consortium, <http://www.chargeconsortium.com/main/exomechip>
Exome Chip Design, http://genome.sph.umich.edu/wiki/Exome_Chip_Design
International HapMap Project, <http://hapmap.ncbi.nlm.nih.gov/>

NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/EVS/>

OMIM, <http://www.omim.org/>

UCSC Genome Browser, <http://genome.ucsc.edu>

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