

Supplemental Data

Regulatory variants in *TCF7L2* are associated with thoracic aortic aneurysm

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Supplementary Information

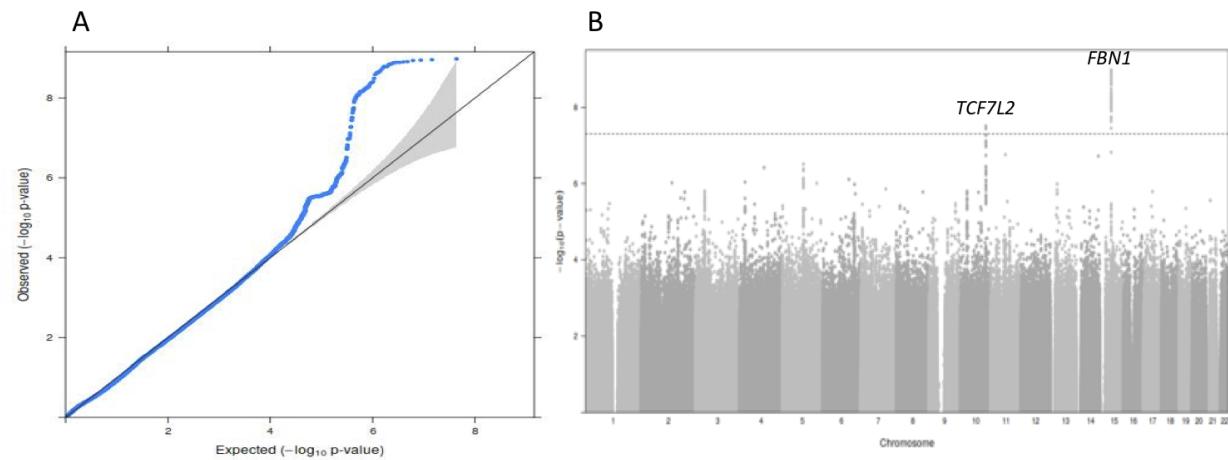


Figure S1: GWAS of TAA in CHIP/MGI. A) QQ plot. B) Manhattan plot.

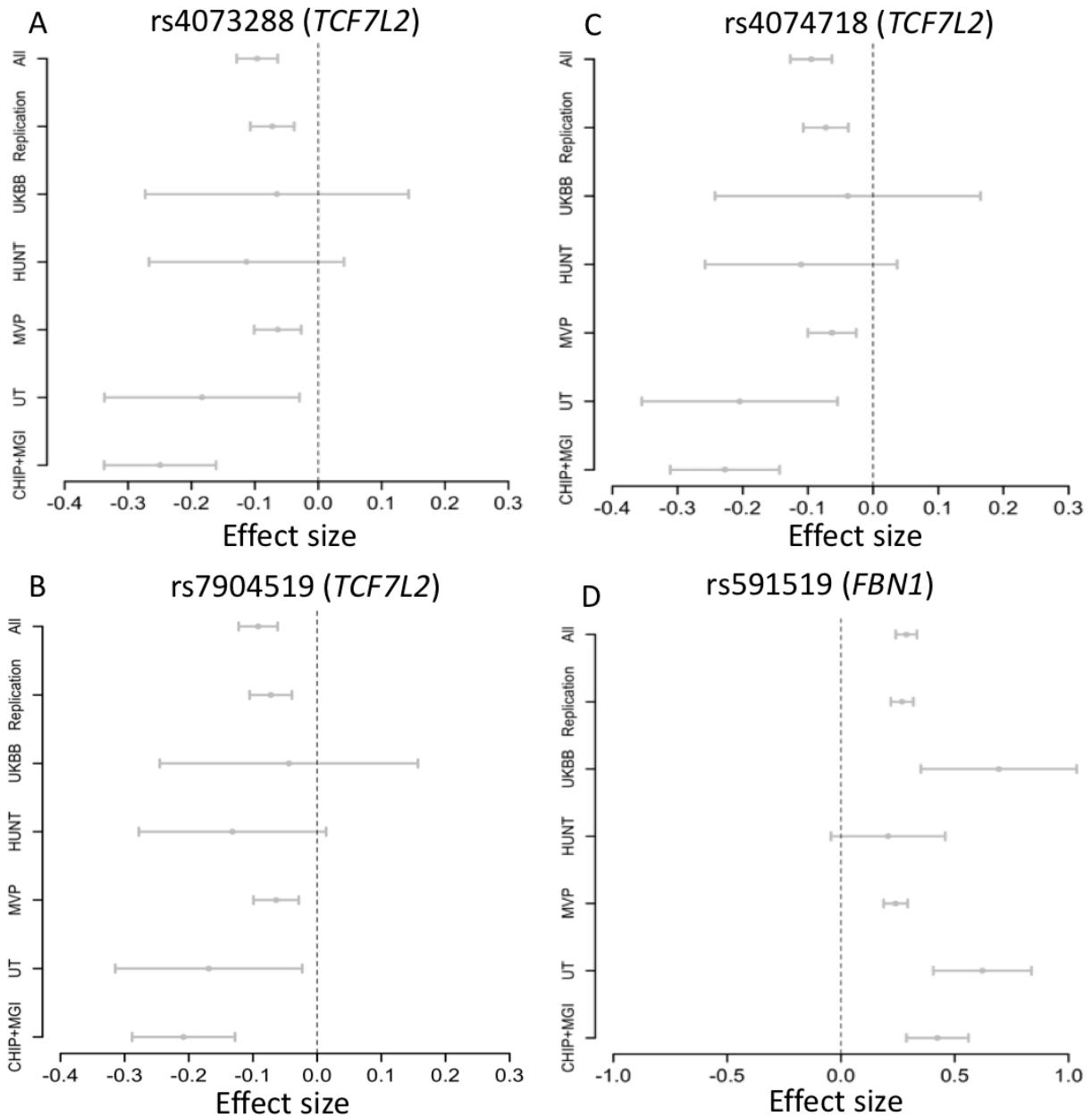


Figure S2: Effect sizes in discovery and replication cohorts. Effect size estimation for 3 variants in *TCF7L2* locus (A-C) and *FBNI* index variant (D) in individual cohorts, replication (UT+MVP+HUNT+UKBB), All (CHIP+MGI+Replication).

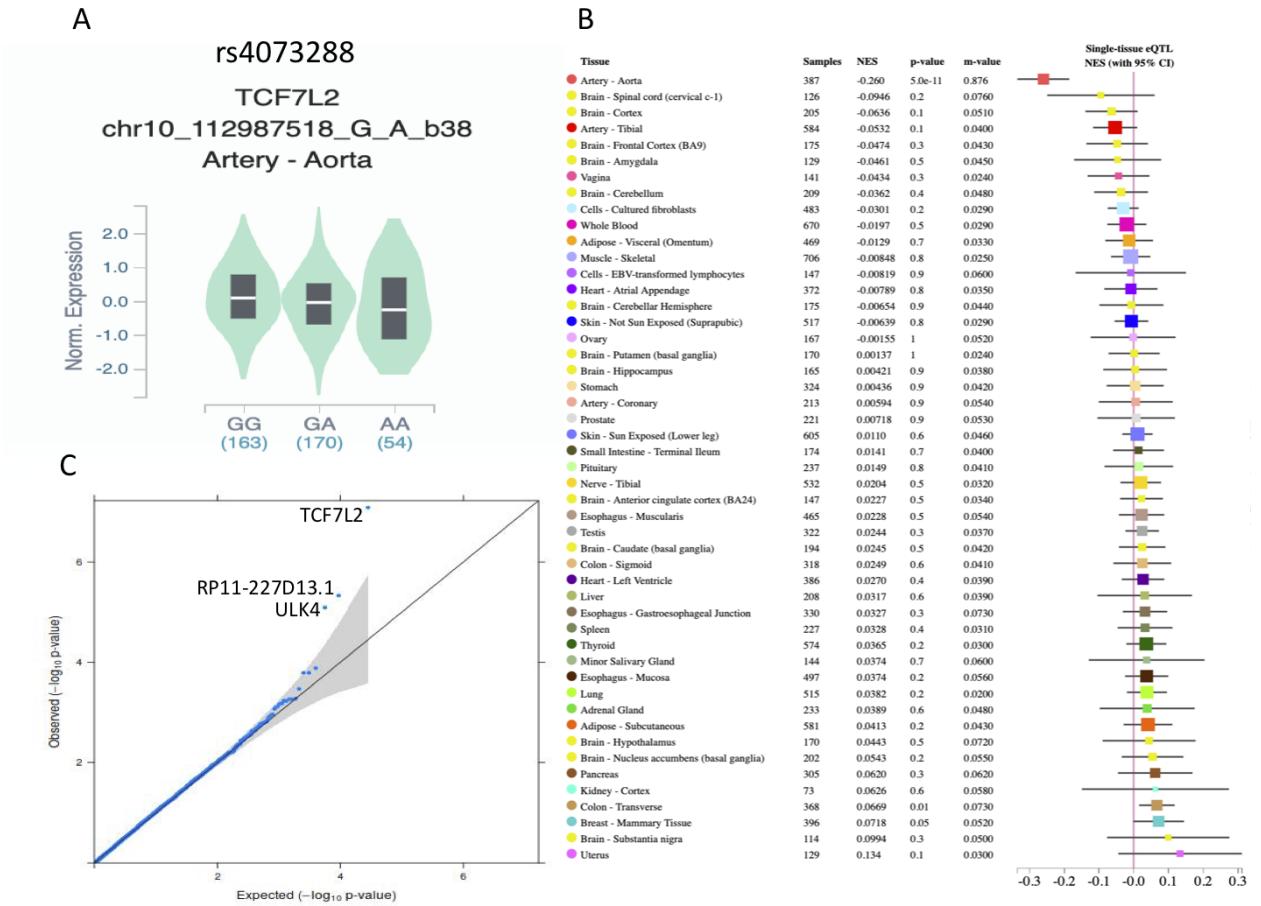


Figure S3: eQTL. **A)** rs4073288 is an aortic eQTL of *TCF7L2* from GTEx V8. TAA risk allele G is associated with higher expression of gene. **B)** This eQTL is only significant in aorta in GTEx V8. **C)** QQ plot of TWAS using SPrediXcan.

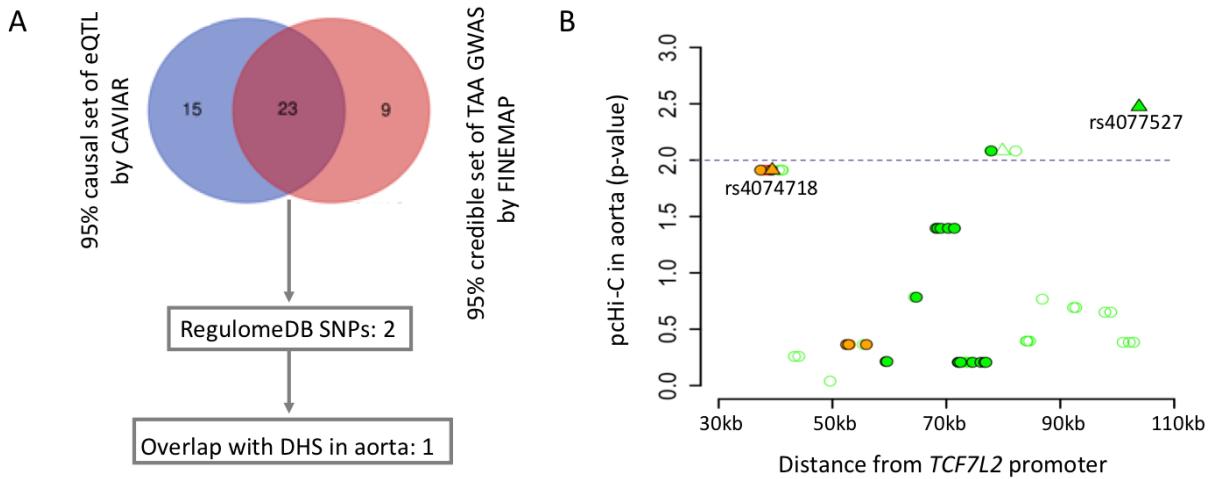


Figure S4: Fine-mapping of *TCF7L2* loci. **A)** 23 variants were found to be common between the credible sets of TAA GWAS and eQTL (rs4073980, rs4074720, rs4074718, rs10885402, rs6585197, rs6585199, rs6585200, rs6585201, rs7904519, rs7918599, rs10885406, rs11196190, rs7899529, rs11196191, rs10787472, rs10787473, rs12258200, rs11196193, rs4309084, rs4128598, rs4128597, rs7907610, rs4077527) **B)** Promoter capture Hi-C -log₁₀(p-values) of variants that are in LD > 0.4 with TAA index variant rs4073980. Solid shapes are 23 variants mentioned in A. Triangles represent variants prioritized by RegulomeDB. The dotted line is the significance threshold as suggested by Jung et al. [PMID: 31501517].

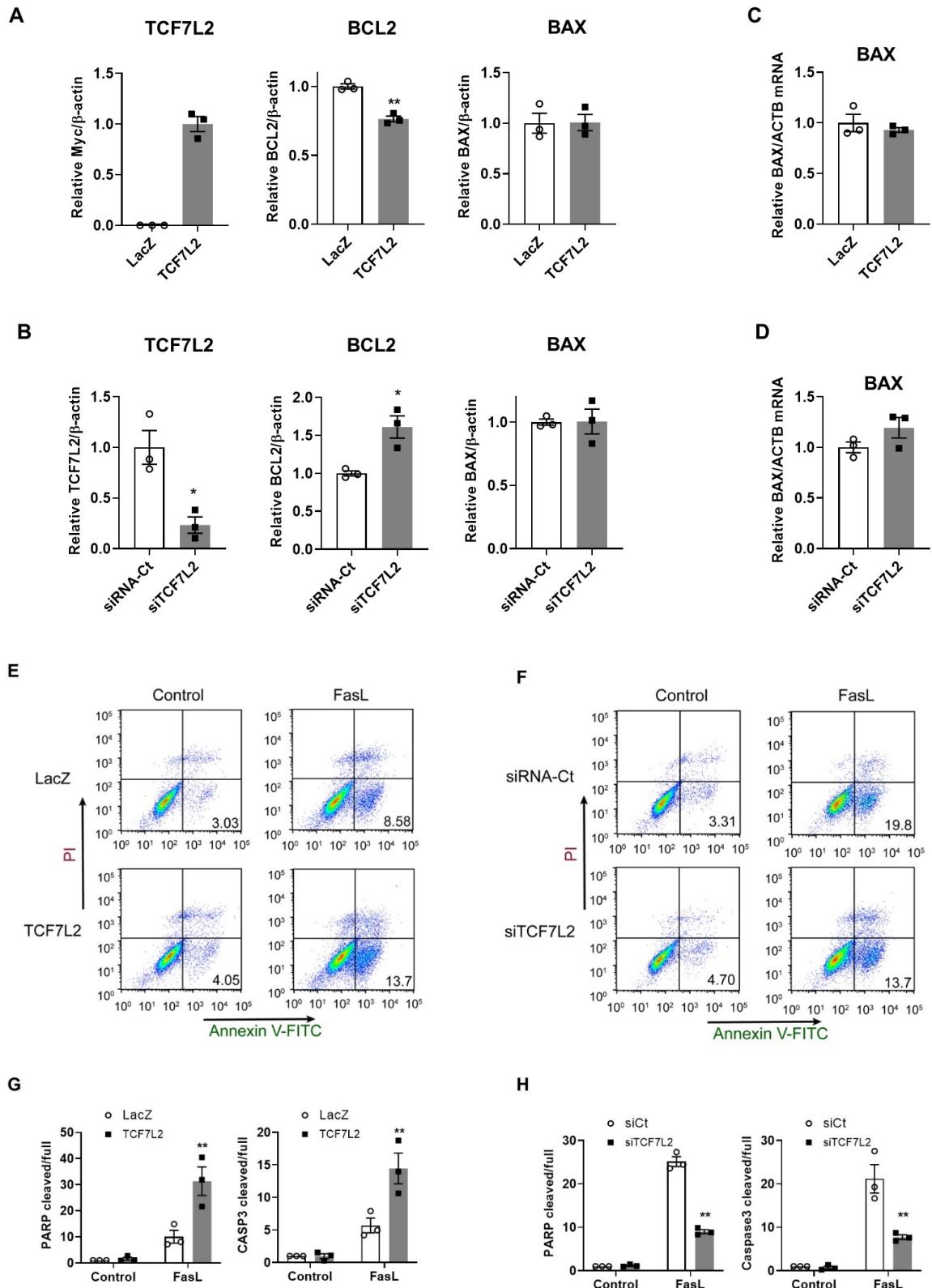


Figure S5. *TCF7L2* expression is associated with vascular smooth muscle cell apoptosis *in vitro*:
A and B, densitometry of 3 independent Western blots as in Figure 3 A and B. **A)** Overexpression of *TCF7L2* showing significant downregulation of *BCL2*, P=0.0011. Densitometry for Myc-*TCF7L2* shows reproducibility among the three independent experiments. **B)** Knockdown of *TCF7L2* (*TCF7L2*, P=0.0145) showing significant upregulation of *BCL2*, P=0.0154. **C)** upregulation of *TCF7L2* or **D)** siRNA-mediated knockdown of *TCF7L2* does not change expression of *BAX* mRNA. Representative graphs of Annexin-V by FACS analysis in response to **E)** upregulation or **F)** knockdown of *TCF7L2*, respectively (n=3; representative of 3 independent experiments). **G and H,** Densitometry of 3 independent Western blots as in Figure 3 F and H. Data are expressed as the ratio between either cleaved PARP or cleaved caspase-3 and its corresponding full length counterpart. **G)** Overexpression of *TCF7L2* combined with FasL treatment: PARP, P=0.0021; Capase3, P=0.0031. **H)** Downregulation of *TCF7L2* combined with FasL treatment: PARP, P<0.0001; Capase3, P=0.0009.

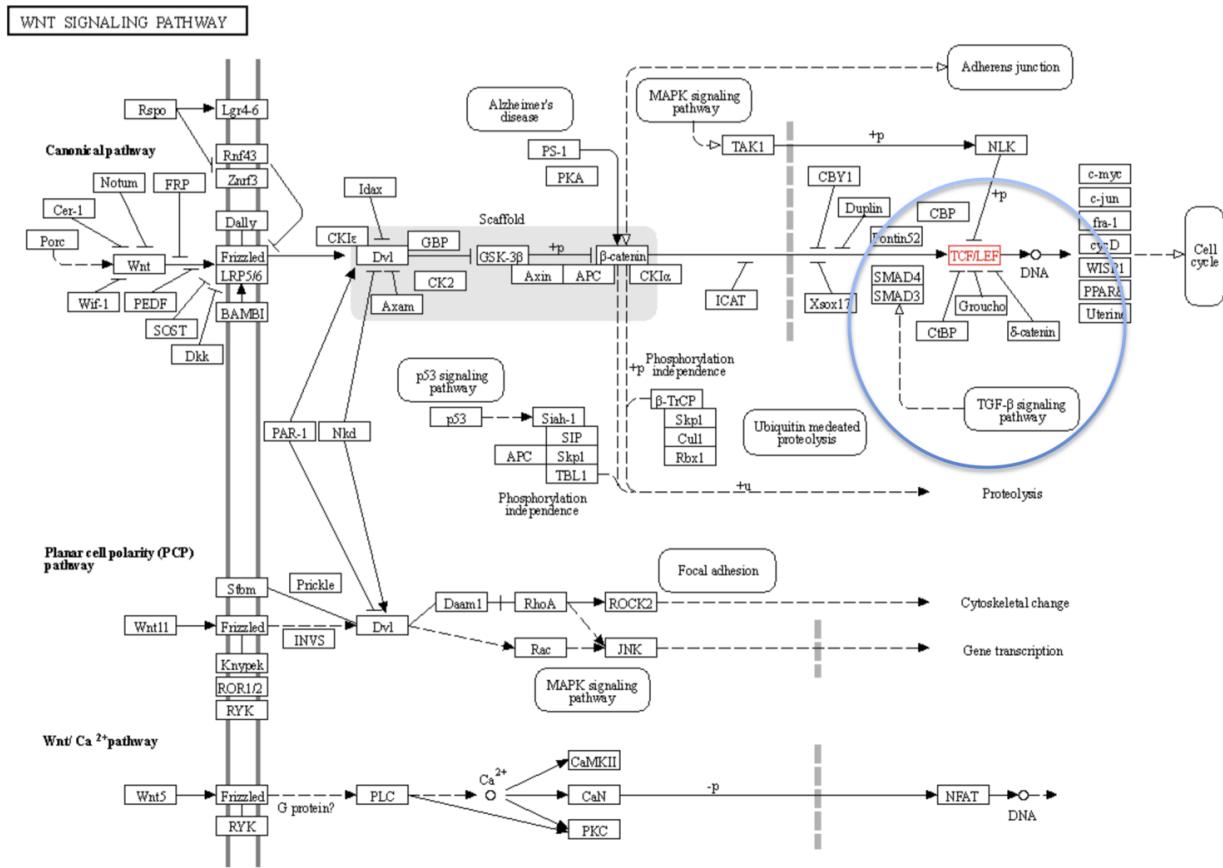


Figure S6: Wnt signaling pathway from KEGG.

Table S1: Characteristics of discovery and replication cohorts

CHIP+MGI	Cases	Controls
Male	71.3%	74%
Median birth year	1948	1954
Present or past smoking*	10.2%	10%
UT**		
Male	65.4%	NA
Median Age	65	NA
Present or past smoking	61.4%	NA
MVP		
Male	97.9%	90.6%
Median age	73	69
HUNT		
Male	64.4%	46.9%
Median birth year	1942	1951
Present or past smoking	64.4%	57.9%
UKBB		
Male	68.1%	68.6%
Median birth year	1945	1945
Present or past smoking	67.7%	64.1%

*Smoking information in MGI was obtained by ICD codes.

**UT data was obtained from supplementary table 1 of LeMaire et al.
(<https://www.nature.com/articles/ng.934#MOESM20>)

Table S2: Codes used for case-control selection in MVP cohort.**TAA CONTROLS****(cannot have any of the following codes, ever)**

- 441 Dissection of aorta, unspecified site
- 441.01 Dissection of aorta, thoracic
- 441.02 Dissection of aorta, abdominal
- 441.03 Dissection of aorta, thoracoabdominal
- 441.1 Thoracic aneurysm, ruptured
- 441.2 Thoracic aneurysm without mention of rupture
- 441.5 Aortic aneurysm of unspecified site, ruptured
- 441.6 Thoracoabdominal aneurysm, ruptured
- 441.7 Thoracoabdominal aneurysm, without mention of rupture
- 441.9 Aortic aneurysm of unspecified site without mention of rupture
- I71.00 Dissection of unspecified site of aorta
- I71.01 Dissection of thoracic aorta
- I71.02 Dissection of abdominal aorta
- I71.03 Dissection of thoracoabdominal aorta
- I71.1 Thoracic aortic aneurysm, ruptured

I71.2	Thoracic aortic aneurysm, without rupture
I71.5	Thoracoabdominal aortic aneurysm, ruptured
I71.6	Thoracoabdominal aortic aneurysm, without rupture
I71.8	Aortic aneurysm of unspecified site, ruptured
I71.9	Aortic aneurysm of unspecified site, without rupture
746.4	Congenital insufficiency of aortic valve
Q23.1	Congenital insufficiency of aortic valve

controls cannot have any of the *_REPAIR codes (ASC_REPAIR, ARCH_REPEAR, DESC_REPAIR, ANY_REPAIR)

controls must also have 2 visits to the VA in each of the two years prior to MVP enrollment

ASC_REPAIR (ascending)

defined as having any of the following CPT codes:

33860	Ascending aortic replacement with valve resuspension when performed Ascending aorta graft, with cardiopulmonary bypass, with or without valve suspension;
33861	with coronary reconstruction
33858	Ascending aortic replacement with valve resuspension when performed (dissection)
33859	Ascending aortic replacement with valve resuspension when performed (non-dissection)
33863	Root replacement (Bentall)
33864	Valve sparing root reconstruction (David)
02QX0ZZ	Repair Thoracic Aorta, Ascending/Arch, Open Approach
02QX3ZZ	Repair Thoracic Aorta, Ascending/Arch, Percutaneous Approach
02QX4ZZ	Repair Thoracic Aorta, Ascending/Arch, Percutaneous Endoscopic Approach Replacement of Thoracic Aorta, Ascending/Arch with Autologous Tissue Substitute,
02RX07Z	Open Approach Replacement of Thoracic Aorta, Ascending/Arch with Zooplastie Tissue, Open
02RX08Z	Approach Replacement of Thoracic Aorta, Ascending/Arch with Synthetic Substitute, Open
02RX0JZ	Approach Replacement of Thoracic Aorta, Ascending/Arch with Nonautologous Tissue Substitute,
02RX0KZ	Open Approach Replacement of Thoracic Aorta, Ascending/Arch with Autologous Tissue Substitute,
02RX47Z	Percutaneous Endoscopic Approach Replacement of Thoracic Aorta, Ascending/Arch with Zooplastie Tissue, Percutaneous
02RX48Z	Endoscopic Approach Replacement of Thoracic Aorta, Ascending/Arch with Synthetic Substitute,
02RX4JZ	Percutaneous Endoscopic Approach Replacement of Thoracic Aorta, Ascending/Arch with Nonautologous Tissue Substitute,
02RX4KZ	Percutaneous Endoscopic Approach

	Restriction of Thoracic Aorta, Ascending/Arch with Intraluminal Device, Open
02VX0DZ	Approach
	Restriction of Thoracic Aorta, Ascending/Arch with Branched or Fenestrated
02VX0EZ	Intraluminal Device, One or Two Arteries, Open Approach
	Restriction of Thoracic Aorta, Ascending/Arch with Branched or Fenestrated
02VX0FZ	Intraluminal Device, Three or More Arteries, Open Approach
02VX0ZZ	Restriction of Thoracic Aorta, Ascending/Arch, Open Approach
	Restriction of Thoracic Aorta, Ascending/Arch with Intraluminal Device, Percutaneous
02VX3DZ	Approach
	Restriction of Thoracic Aorta, Ascending/Arch with Branched or Fenestrated
02VX3EZ	Intraluminal Device, One or Two Arteries, Percutaneous Approach
	Restriction of Thoracic Aorta, Ascending/Arch with Branched or Fenestrated
02VX3FZ	Intraluminal Device, Three or More Arteries, Percutaneous Approach
02VX3ZZ	Restriction of Thoracic Aorta, Ascending/Arch, Percutaneous Approach

ARCH_REPAIR

defined as having any of the following CPT codes:

- 33866 Hemiarch reconstruction
- 33871 Extended arch procedures (more than hemiarch)
- 33870 Transverse arch graft

DESC_REPAIR (descending)

defined as having any of the following CPT codes:

- 33877 Repair TAAA with graft, with or without bypass
- 33875 Repair TAA with graft, with or without bypass
- 33880 TEVAR (Zone 2)
- 33881 TEVAR (not Zone 2)
- 33877 Repair of thoracoadbominal aortic aneurysm with graft |
- 02QW0ZZ Repair Thoracic Aorta, Descending, Open Approach
- 02QW3ZZ Repair Thoracic Aorta, Descending, Percutaneous Approach
- 02QW4ZZ Repair Thoracic Aorta, Descending, Percutaneous Endoscopic Approach
- Replacement of Thoracic Aorta, Descending with Autologous Tissue Substitute, Open
- 02RW07Z Approach
- Replacement of Thoracic Aorta, Descending with Zooplastic Tissue, Open Approach
- 02RW0JZ Replacement of Thoracic Aorta, Descending with Synthetic Substitute, Open Approach
- Replacement of Thoracic Aorta, Descending with Nonautologous Tissue Substitute,
- 02RW0KZ Open Approach
- Replacement of Thoracic Aorta, Descending with Autologous Tissue Substitute,
- 02RW47Z Percutaneous Endoscopic Approach
- Replacement of Thoracic Aorta, Descending with Zooplastic Tissue, Percutaneous
- 02RW48Z Endoscopic Approach

	Replacement of Thoracic Aorta, Descending with Synthetic Substitute, Percutaneous
02RW4JZ	Endoscopic Approach
	Replacement of Thoracic Aorta, Descending with Nonautologous Tissue Substitute,
02RW4KZ	Percutaneous Endoscopic Approach
	Restriction of Thoracic Aorta, Descending with Intraluminal Device, Percutaneous
02VW3DZ	Approach
02VW0DZ	Restriction of Thoracic Aorta, Descending with Intraluminal Device, Open Approach
	Restriction of Thoracic Aorta, Descending with Branched or Fenestrated Intraluminal
02VW0EZ	Device, One or Two Arteries, Open Approach
	Restriction of Thoracic Aorta, Descending with Branched or Fenestrated Intraluminal
02VW0FZ	Device, Three or More Arteries, Open Approach
02VW0ZZ	Restriction of Thoracic Aorta, Descending, Open Approach
	Restriction of Thoracic Aorta, Descending with Branched or Fenestrated Intraluminal
02VW3EZ	Device, One or Two Arteries, Percutaneous Approach
	Restriction of Thoracic Aorta, Descending with Branched or Fenestrated Intraluminal
02VW3FZ	Device, Three or More Arteries, Percutaneous Approach
02VW3ZZ	Restriction of Thoracic Aorta, Descending, Percutaneous Approach
ANY_REPAIR	
defined as having ASC_REPAIR, ARCH_REPAIR, DESC_REPAIR, or having any of the of the codes below:	
38.35 Repair thoracic aorta	
38.45 Resection thoracic aorta	
39.73 TEVAR	

Table S3: Description of chromatin marks from ENCODE.

Mark	ID	Description of source
DNase-seq	ENCSR422IIZ	Ascending aorta of 51 year old female
DNase-seq	ENCSR968TPO	Ascending aorta of 53 year old female
ChIP-seq of H3k27ac	ENCSR318HUC	Thoracic aorta of 54 year old male
ChIP-seq of H3k27ac	ENCSR069UMW	Ascending aorta of 53 year old female

Table S4: Relationship of TAA index variant (rs4073288) with T2D independent variants in *TCF7L2* locus.

T2D independent variants	LD R ² with rs4073288	TAA p-value of rs4073288 after conditioning with T2D variant
rs7903146	0.2846	4.5×10 ⁻⁴
rs536643418	0.0073	2.4×10 ⁻⁸
rs140242150	0.0008	3.5×10 ⁻⁸
rs7918400	0.08	3.1×10 ⁻⁶
rs184509201	0.0004	3.8×10 ⁻⁸
rs180988137	0.0132	6.1×10 ⁻⁸
rs78025551	0.2468	4.5×10 ⁻⁵
rs34855922	0.0203	3.9×10 ⁻⁸

Table S5: Description of RegulomeDB ranks and associated variant count in 95% credible set of *TCF7L2* locus.

RegulomeDB rank	Description	Variant count
3a	TF binding + any motif + DNase peak	2
4	TF binding + DNase peak	4
5	TF binding or DNase peak	12
6	Motif hit	5
7	Others	9

Table S6: Genetic correlation between TAA and other traits as measured by LDSC.

Trait	r _g	S.E.	P	Source/PMID
SBP	-0.0069	0.0813	0.9327	UKBB
DBP	0.2713	0.0916	0.0031	UKBB
Smoking Initiation	0.1966	0.1902	0.3011	30617275
Cig. Per day	0.211	0.1586	0.1833	30617275
Total cholesterol	0.0314	0.1286	0.8072	20686565
HDL	-0.0905	0.1336	0.4983	20686565
LDL	0.0271	0.1565	0.8623	20686565
Triglyceride	0.2149	0.1197	0.0725	20686565
CAD	0.1749	0.108	0.1054	26343387
T2D	0.006	0.147	0.963	22885922
Height	0.3286	0.1139	0.0039	20881960

Table S7: Primers used for qPCR.

Gene	Sequence: 5' to 3'
BCL2	Forward:TCATGTGTGGAGAGCGTC
	Reverse:GCCGTACAGTTCCACAAAGG
BAX	Forward:CCCGAGAGGTCTTTCCGAG
	Reverse:CCAGCCCATGATGGTTCTGAT
TCF7L2	Forward:GAATCGTCCCAGAGTGATGTC
	Reverse:ACGACCTTGCTCTCATTCC
ACTB	Forward:GCTATCACCTCCCTGTGTG
	Reverse:GTCATTCAAATATGAGATGCGT

Table S8: Effect-size variation within discovery cohort.

TCF7L2 (rs7904519)							
	N _{case}	N _{control}	AF _{case}	AF _{control}	OR	95% CI	Chi-square P
CHIP _{cases} /MGI _{controls}	956	18295	0.414	0.473	0.78	0.71-0.86	3.6×10^{-7}
MGI _{cases} /MGI _{controls}	395	18295	0.434	0.473	0.84	0.73-0.97	0.02
CHIP _{AA} /MGI _{controls}	773	18295	0.416	0.473	0.79	0.71-0.87	9.7×10^{-6}
CHIP _{DA} /MGI _{controls}	83	18295	0.349	0.473	0.59	0.43-0.82	0.001
FBNI(rs16961065)							
	N _{case}	N _{control}	AF _{case}	AF _{control}	OR	95% CI	Chi-square P
CHIP _{cases} /MGI _{controls}	956	18295	0.142	0.10	1.48	1.29-1.69	4.1×10^{-9}
MGI _{cases} /MGI _{controls}	395	18295	0.124	0.10	1.27	1.02-1.57	0.02
CHIP _{AA} /MGI _{controls}	773	18295	0.142	0.10	1.48	1.27-1.71	1.1×10^{-7}
CHIP _{DA} /MGI _{controls}	83	18295	0.114	0.10	1.17	0.70-1.84	0.53

CHIP_{AA}: ascending thoracic aneurysm in CHIP; CHIP_{DA}: descending thoracic aneurysm in CHIP

Table S9: Effect-size variation by phenotype definition within MVP cohort.

	EA	TAA N _{case} =6554				Any Thoracic Aortic Repair N _{case} =730				Ascending + Arch Repair N _{case} =444			
		Beta	SE	OR 95% CI	P	Beta	SE	OR 95% CI	P	Beta	SE	OR 95% CI	P
rs4073288 (TCF7L2)	A	-0.06	0.02	0.90-0.98	3.6×10^{-3}	-0.11	0.06	0.8-1	0.05	-0.11	0.07	0.78-1.03	0.14
rs7904519 (TCF7L2)	G	-0.06	0.02	0.90-0.98	4×10^{-4}	-0.12	0.05	0.8-0.98	0.03	-0.13	0.07	0.76-1	0.06
rs4074718 (TCF7L2)	A	-0.06	0.02	0.90-0.98	8×10^{-4}	-0.15	0.06	0.76-0.97	9×10^{-3}	-0.15	0.07	0.75-0.99	0.04
Rs591519 (FBNI)	T	0.24	0.03	1.20-1.35	9×10^{-20}	0.48	0.07	1.41-1.85	1.4×10^{-10}	0.57	0.09	1.48-2.11	4.6×10^{-10}

N_{control}=329971 for all comparisons

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Updated December 10, 2020

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- N. FL/S. GA Veterans Health System (Peruvemba Sriram, M.D.)
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- Northport VA Medical Center (Shing Shing Yeh, Ph.D., M.D.)
79 Middleville Road, Northport, NY 11768
- Overton Brooks VA Medical Center (Neeraj Tandon, M.D.)
510 East Stoner Ave, Shreveport, LA 71101
- Philadelphia VA Medical Center (Darshana Jhala, M.D.)
3900 Woodland Avenue, Philadelphia, PA 19104
- Phoenix VA Health Care System (Samuel Aguayo, M.D.)
650 E. Indian School Road, Phoenix, AZ 85012
- Portland VA Medical Center (David Cohen, M.D.)
3710 SW U.S. Veterans Hospital Road, Portland, OR 97239
- Providence VA Medical Center (Satish Sharma, M.D.)
830 Chalkstone Avenue, Providence, RI 02908
- Richard Roudebush VA Medical Center (Suthat Liangpunsakul, M.D., M.P.H.)
1481 West 10th Street, Indianapolis, IN 46202
- Salem VA Medical Center (Kris Ann Oursler, M.D.)
1970 Roanoke Blvd, Salem, VA 24153

- San Francisco VA Health Care System (Mary Whooley, M.D.)
4150 Clement Street, San Francisco, CA 94121
- South Texas Veterans Health Care System (Sunil Ahuja, M.D.)
7400 Merton Minter Boulevard, San Antonio, TX 78229
- Southeast Louisiana Veterans Health Care System (Joseph Constans, Ph.D.)
2400 Canal Street, New Orleans, LA 70119
- Southern Arizona VA Health Care System (Paul Meyer, M.D., Ph.D.)
3601 S 6th Avenue, Tucson, AZ 85723
- Sioux Falls VA Health Care System (Jennifer Greco, M.D.)
2501 W 22nd Street, Sioux Falls, SD 57105
- St. Louis VA Health Care System (Michael Rauchman, M.D.)
915 North Grand Blvd, St. Louis, MO 63106
- Syracuse VA Medical Center (Richard Servatius, Ph.D.)
800 Irving Avenue, Syracuse, NY 13210
- VA Eastern Kansas Health Care System (Melinda Gaddy, Ph.D.)
4101 S 4th Street Trafficway, Leavenworth, KS 66048
- VA Greater Los Angeles Health Care System (Agnes Wallbom, M.D., M.S.)
11301 Wilshire Blvd, Los Angeles, CA 90073
- VA Long Beach Healthcare System (Timothy Morgan, M.D.)
5901 East 7th Street Long Beach, CA 90822
- VA Maine Healthcare System (Todd Stapley, D.O.)
1 VA Center, Augusta, ME 04330
- VA New York Harbor Healthcare System (Scott Sherman, M.D., M.P.H.)
423 East 23rd Street, New York, NY 10010
- VA Pacific Islands Health Care System (George Ross, M.D.)
459 Patterson Rd, Honolulu, HI 96819
- VA Palo Alto Health Care System (Philip Tsao, Ph.D.)
3801 Miranda Avenue, Palo Alto, CA 94304-1290
- VA Pittsburgh Health Care System (Patrick Strollo, Jr., M.D.)
University Drive, Pittsburgh, PA 15240
- VA Puget Sound Health Care System (Edward Boyko, M.D.)
1660 S. Columbian Way, Seattle, WA 98108-1597
- VA Salt Lake City Health Care System (Laurence Meyer, M.D., Ph.D.)
500 Foothill Drive, Salt Lake City, UT 84148
- VA San Diego Healthcare System (Samir Gupta, M.D., M.S.C.S.)
3350 La Jolla Village Drive, San Diego, CA 92161
- VA Sierra Nevada Health Care System (Mostaqul Huq, Pharm.D., Ph.D.)
975 Kirman Avenue, Reno, NV 89502
- VA Southern Nevada Healthcare System (Joseph Fayad, M.D.)
6900 North Pecos Road, North Las Vegas, NV 89086

- VA Tennessee Valley Healthcare System (Adriana Hung, M.D., M.P.H.)
1310 24th Avenue, South Nashville, TN 37212
- Washington DC VA Medical Center (Jack Lichy, M.D., Ph.D.)
50 Irving St, Washington, D. C. 20422
- W.G. (Bill) Hefner VA Medical Center (Robin Hurley, M.D.)
1601 Brenner Ave, Salisbury, NC 28144
- White River Junction VA Medical Center (Brooks Robey, M.D.)
163 Veterans Drive, White River Junction, VT 05009
- William S. Middleton Memorial Veterans Hospital (Robert Striker, M.D., Ph.D.)
2500 Overlook Terrace, Madison, WI 53705