

# Genome-wide association study identifies a susceptibility locus for thoracic aortic aneurysms and aortic dissections spanning *FBN1* at 15q21.1

Scott A LeMaire<sup>1,2,17</sup>, Merry-Lynn N McDonald<sup>3,17</sup>, Dong-chuan Guo<sup>4,17</sup>, Ludivine Russell<sup>1,2</sup>, Charles C Miller III<sup>5</sup>, Ralph J Johnson<sup>4</sup>, Mir Reza Bekheirnia<sup>3</sup>, Luis M Franco<sup>3</sup>, Mary Nguyen<sup>1,2</sup>, Reed E Pyeritz<sup>6</sup>, Joseph E Bavaria<sup>7</sup>, Richard Devereux<sup>8</sup>, Cheryl Maslen<sup>9</sup>, Kathryn W Holmes<sup>10</sup>, Kim Eagle<sup>11</sup>, Simon C Body<sup>12</sup>, Christine Seidman<sup>13</sup>, J G Seidman<sup>13</sup>, Eric M Isselbacher<sup>14</sup>, Molly Bray<sup>15</sup>, Joseph S Coselli<sup>1,2</sup>, Anthony L Estrera<sup>5</sup>, Hazim J Safi<sup>5</sup>, John W Belmont<sup>3</sup>, Suzanne M Leal<sup>3</sup> & Dianna M Milewicz<sup>4,16</sup>

Although thoracic aortic aneurysms and dissections (TAAD) can be inherited as a single-gene disorder, the genetic predisposition in the majority of affected people is poorly understood. In a multistage genome-wide association study (GWAS), we compared 765 individuals who had sporadic TAAD (STAAD) with 874 controls and identified common SNPs at a 15q21.1 locus that were associated with STAAD, with odds ratios of 1.6–1.8 that achieved genome-wide significance. We followed up 107 SNPs associated with STAAD with  $P < 1 \times 10^{-5}$  in the region, in two separate STAAD cohorts. The associated SNPs fall into a large region of linkage disequilibrium encompassing *FBN1*, which encodes fibrillin-1. *FBN1* mutations cause Marfan syndrome, whose major cardiovascular complication is TAAD. This study shows that common genetic variants at 15q21.1 that probably act via *FBN1* are associated with STAAD, suggesting a common pathogenesis of aortic disease in Marfan syndrome and STAAD.

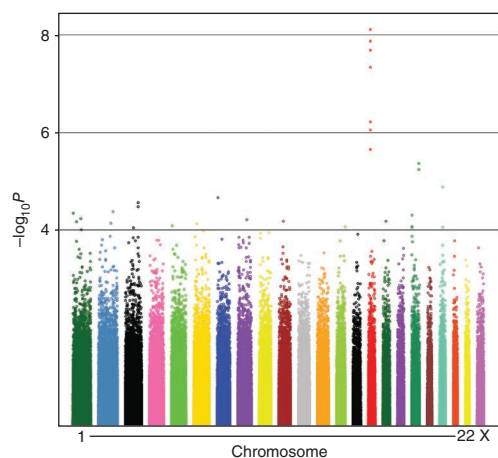
The natural history of aneurysms involving the aortic root and ascending thoracic aorta is progressive, asymptomatic enlargement over time, ultimately leading to life-threatening acute ascending aortic dissection (termed type A dissection in the Stanford classification) or rupture. Less deadly aortic dissections can originate in the descending thoracic aorta just distal to the origin of the left subclavian artery (type B dissections). All of these conditions are part of the TAAD

disease spectrum. Risk factors for TAAD include poorly controlled hypertension and congenital cardiovascular abnormalities, such as a bicuspid aortic valve (BAV) and aortic coarctation. In addition, genetic predisposition has a prominent role in the etiology of TAAD. Thoracic aortic disease is inherited in families in an autosomal-dominant manner in the presence or absence of syndromic features. Marfan syndrome is an example of a genetic syndrome in which essentially all affected individuals have TAAD, in addition to skeletal and ocular complications<sup>1</sup>. Marfan syndrome results from heterozygous mutations in *FBN1*, which encodes an extracellular matrix protein (fibrillin-1) that is a component of the elastic fibers in the medial layer of the aorta.

Family aggregation studies indicate that up to one-fifth of individuals with TAAD who lack features of a genetic syndrome have family histories of TAAD; in these cases, TAAD is also inherited in an autosomal-dominant manner. The underlying genetic heterogeneity in familial TAAD results in substantial clinical heterogeneity in aortic disease presentation and associated vascular diseases; to date, seven genes have been identified that account for 20% of familial TAAD<sup>2–5</sup>.

Although most TAAD is sporadic, the genetic basis of STAAD has not been fully explored. We performed a three-stage GWAS. For stage 1, we used samples from 765 affected individuals of European descent who presented for treatment of an ascending thoracic aortic aneurysm and/or a type A or B aortic dissection, who were more than

<sup>1</sup>Division of Cardiothoracic Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, Texas, USA. <sup>2</sup>Cardiovascular Surgery, Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas, USA. <sup>3</sup>Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA. <sup>4</sup>Division of Medical Genetics, Department of Internal Medicine, University of Texas Health Science Center at Houston, Houston, Texas, USA. <sup>5</sup>Department of Cardiothoracic and Vascular Surgery, University of Texas Health Science Center at Houston, Houston, Texas, USA. <sup>6</sup>Division of Medical Genetics, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. <sup>7</sup>Division of Cardiovascular Surgery, Department of Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. <sup>8</sup>Greenberg Division of Cardiology, Department of Medicine, Weill Cornell Medical College, New York, New York, USA. <sup>9</sup>Division of Cardiovascular Medicine, Department of Molecular & Medical Genetics, Oregon Health & Science University, Portland, Oregon, USA. <sup>10</sup>Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. <sup>11</sup>Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA. <sup>12</sup>Department of Anesthesiology, Harvard Medical School, Boston, Massachusetts, USA. <sup>13</sup>Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA. <sup>14</sup>Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA. <sup>15</sup>Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA. <sup>16</sup>Medicine Services, Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas, USA. <sup>17</sup>These authors contributed equally to this work. Correspondence should be addressed to D.M.M. (dianna.m.milewicz@uth.tmc.edu).



**Figure 1** A Manhattan plot of stage 1 genome-wide association results from comparison of STAAD cases to NINDS controls. For each tested marker, the significance is displayed on the y-axis as the  $-\log_{10}$  of the  $P$  value. The  $-\log_{10}$  results are ordered along the x-axis by chromosome, with each colored bar representing a different chromosome.

30 years old, and who had no family history of TAAO or evidence of a syndromic form of TAAO on examination (**Supplementary Table 1**). The samples were genotyped with Illumina CNV370-Quad BeadChip arrays, and also analyzed were 1,355 controls from the Wellcome Trust Case-Control Consortium (WTCCC) 1958 Birth Cohort (C58), as well as 874 controls whose data were obtained from the US National Institute of Neurological Disorders and Stroke (NINDS) Repository's Neurologically Normal Control Collection.

Only one locus, at chromosome 15q21.1, harbored SNPs that were associated with STAAD with a genome-wide significance (GWS) level of  $P < 5 \times 10^{-8}$  (**Fig. 1**, **Table 1** and **Supplementary Table 2**). Five SNPs were associated with an increased risk of disease after adjustment for sex and population substructure; odds ratios (ORs) ranged from 1.4 to 1.8. To confirm that our findings were not biased by the different sources of cases and controls, we showed that rs2118181 was associated with STAAD with GWS whether the NINDS ( $P_{\text{stage 1}} = 4.6 \times 10^{-8}$ ) or the C58 ( $P_{\text{stage 1}} = 9.4 \times 10^{-9}$ ) controls were used. The STAAD cases had less evidence of population substructure with the NINDS controls ( $\lambda_{\text{sex, CI}} = 1.005$ ) than with the C58 controls ( $\lambda_{\text{sex, CI}} = 1.06$ ), so we report the results that were obtained using the NINDS controls.

To validate these findings, we genotyped the five GWS 15q21.1 SNPs, along with 99 imputed SNPs and three genotyped 15q21.1 SNPs that were associated ( $P_{\text{stage 1}} < 1 \times 10^{-5}$ ) in two independent STAAD cohorts (stages 2 and 3). Stage 2 comprised 385 individuals with STAAD, 192

(49.9%) of whom also had BAV, and 159 controls (**Supplementary Table 1**). Stage 3 comprised 163 people with sporadic nondissection ascending aortic aneurysms, 157 (96.3%) of whom had BAV, and 476 controls (**Supplementary Table 1**). In both replication stages, all five GWS SNP-STAAD associations replicated with no evidence of heterogeneity between the stages (**Table 1**). Of the 99 imputed SNPs, 62 SNPs were associated with STAAD with GWS in stage 1. In stages 2 and 3, the association was replicated ( $P < 0.05$ ) for 51 of these imputed SNPs (**Fig. 2a** and **Supplementary Table 3**). The meta-analysis of data from stages 1, 2 and 3 identified rs2118181 as the stage 1-genotyped SNP that was most highly associated with STAAD ( $OR_{\text{meta}} = 1.8$ ,  $P_{\text{meta}} = 5.9 \times 10^{-12}$ ); of the imputed SNPs, rs1036476 was most highly associated with STAAD ( $OR_{\text{meta}} = 1.9$ ,  $P_{\text{meta}} = 5.9 \times 10^{-13}$ ; **Supplementary Table 3**). An association plot of the 15q21.1 region showed that the associated SNPs fall into a large linkage disequilibrium region approximately 305 kb in size that encompasses the entire *FBN1* gene, and *FBN1* is the only gene in this linkage disequilibrium region. *FBN1* mutations that cause Marfan syndrome are typically private, rare variants that lead to missense and nonsense mutations and splicing errors. In contrast, the association between 15q21.1 and STAAD is probably mediated by one or more common variants within *FBN1*.

The individuals with STAAD had a spectrum of thoracic aortic disease presentations. A subset had BAV, the most common congenital heart defect, which is found in 1% to 2% of the general population<sup>6</sup>. Among patients referred for surgical treatment of BAV, 20% have concurrent ascending aortic aneurysms<sup>7,8</sup>, and approximately 15% of patients with acute aortic dissections have BAV<sup>9</sup>, indicating a strong association between BAV and risk for STAAD. We investigated whether the 15q21.1 SNPs are associated with STAAD in the presence or absence of BAV. In stages 1 and 2, all five GWS 15q21.1 SNPs were associated with TAAO without BAV. This association was strongest for rs1036477 and rs2118181, but met GWS for all five SNPs in the meta-analysis ( $OR_{\text{no BAV, meta}} = 1.9$ ,  $P_{\text{no BAV, meta}} = 9.9 \times 10^{-10}$ ; **Table 2** and **Supplementary Table 4**). Similarly, 16 imputed 15q21.1 SNPs were associated (meeting GWS) with STAAD without BAV in stage 1, and the associations were replicated in stage 2. Meta-analysis identified rs1036476 as the SNP most significantly associated with STAAD in the absence of BAV ( $OR_{\text{no BAV, meta}} = 2.0$ ,  $P_{\text{no BAV, meta}} = 3.3 \times 10^{-10}$ ; **Supplementary Table 5** and **Supplementary Fig. 1a**).

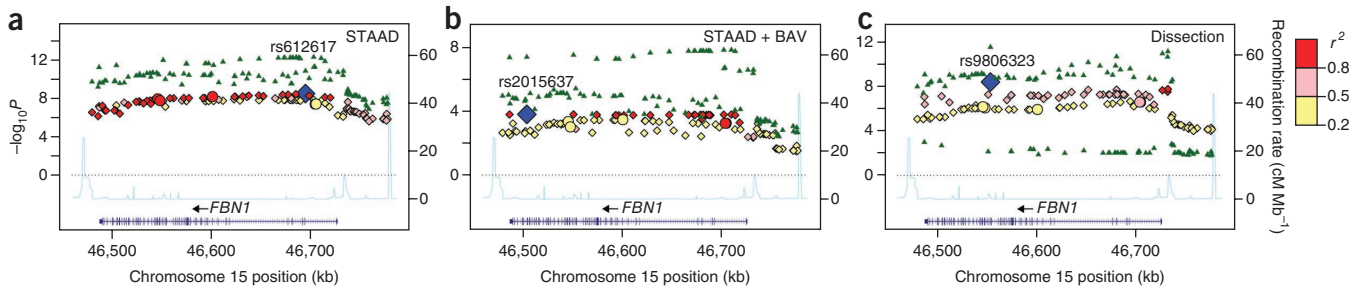
When people with STAAD and BAV were compared to controls, a subset of the five 15q21.1 GWS SNPs were significantly associated in stages 1, 2 and 3; rs2118181 had the most significant association in the meta-analysis ( $OR_{\text{BAV, meta}} = 1.8$ ,  $P_{\text{BAV, meta}} = 2.2 \times 10^{-7}$ ; **Table 2** and **Supplementary Table 4**). When the imputed SNPs were analyzed in stage 1, none of the 99 SNPs was associated with STAAD and BAV with GWS; however, in the meta-analysis, 20 of the SNPs were associated with STAAD and BAV with GWS, the most significant

**Table 1** Genotyped SNPs associated with STAAD ( $P < 5 \times 10^{-8}$ ) with variants within and flanking the *FBN1* gene in stage 1 samples

SNP	BP	Allele	Stage 1 sample <sup>a</sup>				Stage 2 sample <sup>b</sup>				Stage 3 sample <sup>c</sup>				Stages 1, 2 and 3 combined <sup>d</sup>		
			MAF	OR	SE	$P$	MAF	OR	SE	$P$	MAF	OR	SE	$P$	OR	$P$	$Q$
			(765 cases, 874 NINDS controls)				(385 cases, 150 controls)				(163 cases, 476 controls)				(1,313 cases, 1,500 controls)		
rs10519177	46544486	G	0.27	1.6	0.08	$1.3 \times 10^{-8}$	0.27	1.4	0.16	0.02	0.26	1.5	0.15	0.0064	1.6	$2.6 \times 10^{-11}$	0.80
rs4774517	46546582	A	0.27	1.6	0.08	$2.0 \times 10^{-8}$	0.27	1.4	0.16	0.02	0.26	1.5	0.15	0.0064	1.5	$3.8 \times 10^{-11}$	0.82
rs755251	46599311	G	0.27	1.6	0.08	$7.8 \times 10^{-9}$	0.27	1.4	0.16	0.03	0.26	1.5	0.15	0.009	1.6	$3.2 \times 10^{-11}$	0.69
rs1036477	46702217	G	0.13	1.8	0.11	$4.6 \times 10^{-8}$	0.13	2.6	0.26	$2.9 \times 10^{-4}$	0.13	1.6	0.18	0.0103	1.8	$6.5 \times 10^{-12}$	0.30
rs2118181	46703175	G	0.13	1.8	0.11	$4.6 \times 10^{-8}$	0.13	2.6	0.26	$2.9 \times 10^{-4}$	0.13	1.6	0.18	0.0095	1.8	$5.9 \times 10^{-12}$	0.30

MAF, minor allele frequency; OR, odds ratio; SE, standard error of the odds ratio; MDS, multidimensional scaling. BP denotes NCBI Build 36.1 SNP physical position.  $Q$  denotes  $P$  value for the Cochran  $Q$  statistic for homogeneity between stages 1 and 2.

<sup>a</sup>Adjusted for sex and first MDS component from stage 1 samples and NINDS controls. <sup>b</sup>Adjusted for sex and first MDS component from stage 2 samples and controls. <sup>c</sup>Adjusted for sex for stage 3 samples and controls. <sup>d</sup>Fixed-effects meta-analysis results from stage 1 cases and NINDS controls, and from stage 2 and stage 3 samples. For  $Q < 0.1$ , the random effects OR and  $P$  are reported.



**Figure 2** *FBN1* regional association plots. (a–c) Association plots are shown for STAAD (a), STAAD with BAV (b) and aortic dissection (c). The top-ranked stage 1 SNP for each phenotype is marked by a blue diamond. Red, pink and yellow shading denotes  $r^2$  (see key), corresponding to the linkage disequilibrium between the top-ranked SNP and other SNPs in the region. The recombination rate according to HapMap CEU is plotted as a light blue line, with amplitude scaled to the right-hand y-axis. Circles denote the five SNPs genotyped in the *FBN1* region that were associated with TAAD in stage 1 with GWS. Dotted lines denote  $-\log_{10} P = 0$ . Green triangles denote the fixed-effects meta-analysis results for the combined stages. When the result of the Cochran  $Q$  test of homogeneity was  $<0.1$ , random-effects meta-analysis results are shown; otherwise fixed-effects meta-analysis was performed.

being rs689304 ( $OR_{BAV, meta} = 2.0$ ,  $P_{BAV, meta} = 1.7 \times 10^{-8}$ ; **Fig. 2b** and **Supplementary Table 6**). These data support the conclusion that the 15q21.1 locus confers susceptibility to STAAD whether or not the individual has BAV.

The spectrum of STAAD presentation included patients with ascending aortic aneurysms, who are typically referred for surgical repair when the aortic diameter reaches 5.5 cm or more<sup>10</sup>, as well as patients who presented with type A or B aortic dissections. To determine whether the 15q21.1 locus was associated with both disease presentations, we initially analyzed the five GWS 15q21.1 SNPs in people with nondissection aneurysms (NDA) involving the root and/or ascending aorta. The SNPs rs1036477 and rs2118181 were the most significantly associated with such aneurysms ( $OR_{NDA, stage 1} = 1.7$ ,  $P_{NDA, stage 1} = 7.4 \times 10^{-5}$ ;

**Table 2** and **Supplementary Table 4**). Several of these associations were replicated in stages 2 and 3, and in the meta-analysis the SNP most significantly associated with aneurysm was rs2118181 ( $OR_{NDA, meta} = 1.7$ ,  $P_{NDA, meta} = 1.3 \times 10^{-7}$ ). Further investigation of the imputed SNPs in patients who presented with ascending aortic aneurysms revealed 13 SNPs that achieved GWS after meta-analysis of stages 1, 2 and 3; the SNP most significantly associated with nondissection aneurysm was rs636178 ( $OR_{NDA, meta} = 1.7$ ,  $P_{NDA, meta} = 3.5 \times 10^{-8}$ ; **Supplementary Table 7** and **Supplementary Fig. 1b**).

Similarly, in stage 1, the five GWS 15q21.1 SNPs were associated with aortic dissection (AD;  $OR_{AD, stage 1} = 1.9$ ,  $P_{AD, stage 1} = 2.7 \times 10^{-7}$ ). This association was replicated for all five SNPs in stage 2 ( $OR_{AD, stage 2} = 4.1$ ,  $P_{AD, stage 2} = 4.2 \times 10^{-6}$ ); the most significant meta-analysis

**Table 2** Associations between STAAD subphenotypes and genotyped SNPs associated with STAAD ( $P < 5 \times 10^{-8}$ ) with variants within and flanking the *FBN1* gene in stage 1 samples<sup>a</sup>

SNP	BP	Allele	Stage 1 sample <sup>b</sup>				Stage 2 sample <sup>c</sup>				Stage 3 sample <sup>d</sup>				Stages 1, 2 and 3 combined <sup>e</sup>		
			MAF	OR	SE	<i>P</i>	MAF	OR	SE	<i>P</i>	MAF	OR	SE	<i>P</i>	OR	<i>P</i>	<i>Q</i>
<b>STAAD without BAV</b>			<b>(618 cases, 874 NINDS controls)</b>				<b>(193 cases, 150 controls)</b>				<b>(6 cases, 476 controls)</b>				<b>(811 cases, 1,024 controls)</b>		
rs10519177	46544486	G	0.27	1.6	0.09	$7.8 \times 10^{-8}$	0.28	1.6	0.18	$7.5 \times 10^{-3}$	–	–	–	–	1.6	$2.0 \times 10^{-9}$	0.98
rs2118181	46703175	G	0.13	1.8	0.11	$2.3 \times 10^{-7}$	0.12	2.7	0.28	$4.1 \times 10^{-4}$	–	–	–	–	1.9	$9.9 \times 10^{-10}$	0.17
<b>STAAD with BAV</b>			<b>(147 cases, 874 NINDS controls)</b>				<b>(192 cases, 150 controls)</b>				<b>(157 cases, 476 controls)</b>				<b>(339 cases, 1,024 controls)</b>		
rs10519177	46544486	G	0.24	1.6	0.14	$5.0 \times 10^{-4}$	0.25	1.3	0.19	0.19	0.26	1.5	0.15	0.008	1.5	$8.9 \times 10^{-6}$	0.57
rs2118181	46703175	G	0.11	1.8	0.18	$6.3 \times 10^{-4}$	0.11	2.9	0.31	$7.4 \times 10^{-4}$	0.13	1.6	0.18	0.011	1.8	$2.2 \times 10^{-7}$	0.26
<b>Nondissection aneurysm</b>			<b>(401 cases, 874 NINDS controls)</b>				<b>(253 cases, 150 controls)</b>				<b>(163 cases, 476 controls)</b>				<b>(817 cases, 1,500 controls)</b>		
rs10519177	46544486	G	0.26	1.3	0.1	$1.3 \times 10^{-5}$	0.24	1.1	0.17	0.43	0.26	1.5	0.15	0.0064	1.4	$6.2 \times 10^{-7}$	0.32
rs2118181	46703175	G	0.12	1.7	0.13	$7.4 \times 10^{-5}$	0.1	1.9	0.28	0.016	0.13	1.6	0.18	0.0095	1.7	$1.3 \times 10^{-7}$	0.83
<b>Dissection</b>			<b>(364 cases, 874 NINDS controls)</b>				<b>(132 cases, 150 controls)</b>				<b>(0 cases, 476 controls)</b>				<b>(496 cases, 1,024 controls)</b>		
rs10519177	46544486	G	0.26	1.7	0.1	$7.2 \times 10^{-7}$	0.29	2.2	0.2	$1.2 \times 10^{-4}$	–	–	–	–	1.8	$6.6 \times 10^{-10}$	0.26
rs2118181	46703175	G	0.13	1.9	0.13	$2.7 \times 10^{-7}$	0.13	4.1	0.31	$4.2 \times 10^{-6}$	–	–	–	–	2.7	$7.4 \times 10^{-3}$	0.03
<b>Type A dissection</b>			<b>(224 cases, 874 NINDS controls)</b>				<b>(84 cases, 150 controls)</b>				<b>(0 cases, 476 controls)</b>				<b>(308 cases, 1,024 controls)</b>		
rs10519177	46544486	G	0.25	1.7	0.12	$6.1 \times 10^{-6}$	0.28	2.2	0.22	$3.1 \times 10^{-4}$	–	–	–	–	1.8	$1.2 \times 10^{-8}$	0.33
rs2118181	46703175	G	0.12	2.1	0.15	$7.7 \times 10^{-7}$	0.13	5.3	0.33	$4.5 \times 10^{-7}$	–	–	–	–	3.2	$1.4 \times 10^{-2}$	0.01
<b>Type B dissection</b>			<b>(144 cases, 874 NINDS controls)</b>				<b>(53 cases, 150 controls)</b>				<b>(0 cases, 476 controls)</b>				<b>(197 cases, 1,024 controls)</b>		
rs10519177	46544486	G	0.24	1.6	0.15	$8.0 \times 10^{-4}$	0.25	1.8	0.26	0.028	–	–	–	–	1.7	$6.4 \times 10^{-5}$	0.79
rs2118181	46703175	G	0.11	1.8	0.18	$1.4 \times 10^{-3}$	0.08	2	0.4	0.079	–	–	–	–	1.8	$2.8 \times 10^{-4}$	0.78

MAF, minor allele frequency; BAV, bicuspid aortic valve; MDS, multidimensional scaling. BP denotes NCBI Build 36.1 SNP physical position. – denotes that SNP was not analyzed in stage 3 cases without BAV because of low sample size. *Q* denotes *P* value for the Cochran statistic for homogeneity between stages 1 and 2 or stages 1, 2 and 3, as appropriate.

<sup>a</sup>Results for two of the five GWS 15q21.1 SNPs (see **Supplementary Table 4** for the other three SNPs). <sup>b</sup>Adjusted for sex and first MDS component from stage 1 samples and NINDS controls.

<sup>c</sup>Adjusted for sex and first MDS component from stage 2 samples. <sup>d</sup>Adjusted for sex for stage 3 samples. <sup>e</sup>Fixed-effects meta-analysis results between stage 1 and 2 samples for STAAD without BAV, aortic dissection (including type A and type B), and between stage 1, 2 and 3 samples for STAAD, STAAD with BAV, and nondissection aneurysm. Stage 3 aortic dissection (including type A and type B) and STAAD without BAV were not analyzed because there were only a limited number of cases with these subphenotypes. For  $Q < 0.1$ , the random effects OR and *P* are reported.



result was  $OR_{AD, \text{stage } 2} = 1.8$ ,  $P_{AD, \text{stage } 2} = 6.6 \times 10^{-10}$  (Table 2 and Supplementary Table 4) for rs10519177. The OR in stage 2 for rs1036477 and rs2118181 was more than twice the OR in stage 1, and the null hypothesis of homogeneity was rejected ( $P = 0.03$ ). This heterogeneity between stages may reflect differing linkage disequilibrium patterns between the tagged SNPs and the causal variant(s) or differences in the phenotype, or it may be due to chance. For SNPs failing the null hypothesis of homogeneity, a random-effects model was used to combine the stages. A total of nine SNPs were associated with aortic dissection with GWS in stage 1, including the genotyped SNP rs2289136. This result was replicated in stage 2, in which rs9806323 was the SNP most significantly associated with dissection in the meta-analysis ( $OR_{AD, \text{meta}} = 2.1$ ,  $P_{AD, \text{meta}} = 2.9 \times 10^{-12}$ ; Fig. 2c and Supplementary Table 8). Thus, the 15q21.1 locus is associated with presentation with an aneurysm or aortic dissection but is more significantly associated with aortic dissection.

We also sought to determine whether the 15q21.1 locus was associated with aortic dissections originating in the ascending aorta (type A) and descending aorta (type B). The SNPs most significantly associated with type A aortic dissection in stages 1 and 2 were rs1036477 and rs2118181 ( $OR_{AD, A, \text{stage } 1} = 2.1$ ,  $P_{AD, A, \text{stage } 1} = 7.7 \times 10^{-7}$ ;  $OR_{AD, A, \text{stage } 2} = 5.3$ ,  $P_{AD, A, \text{stage } 2} = 4.5 \times 10^{-7}$ ). In the meta-analysis of the type A dissection data from stages 1 and 2, rs10519177 was the most significant SNP ( $OR_{AD, A, \text{meta}} = 1.8$ ,  $P_{AD, A, \text{meta}} = 1.2 \times 10^{-8}$ ; Table 2 and Supplementary Table 4). The null hypothesis of homogeneity between stages 1 and 2 was rejected ( $P = 0.0094$ ) for rs1036477 and rs2118181; therefore, the random-effects model was used to combine the stages. Among the 99 SNPs that were genotyped after being imputed in stage 1, rs9806323 was the one most significantly associated with dissection in the meta-analysis ( $OR_{AD, A, \text{meta}} = 2.4$ ,  $P_{AD, A, \text{meta}} = 4.9 \times 10^{-13}$ ; Supplementary Table 9 and Supplementary Fig. 1c). Although several SNPs were associated with type B dissection in stages 1 and 2, the meta-analysis of the 15q GWS SNPs and imputed SNPs did not indicate GWS for the most significant SNP, rs682938 ( $OR_{AD, B, \text{meta}} = 1.7$ ,  $P_{AD, B, \text{meta}} = 2.0 \times 10^{-5}$ ; Table 2, Supplementary Tables 4 and 10 and Supplementary Fig. 1d). Although the 15q21.1 locus was associated with type A dissection, the association with type B dissection did not reach GWS, possibly because there were fewer type B dissection cases.

We have shown that 15q21.1 SNPs lying in a linkage-disequilibrium region containing the entire *FBN1* gene are associated with STAAD. The 15q21.1 locus association remains robust across various STAAD subphenotypes, including STAAD occurring in the presence or absence of BAV, and presentation with either an aneurysm or type A aortic dissection. It is unlikely that the associations identified in this study were caused by a synthetic association with multiple rare variants, as this would require that these rare variants fall on the same haplotype in all three stages of the study. More likely, the association is due to one or more common variants in *FBN1* that alter fibrillin-1 expression or function. Previous studies have established that *FBN1* mutations can predispose individuals to TAAD in the absence of other phenotypic manifestations of Marfan syndrome<sup>11,12</sup>; therefore, it is not surprising that the possible functional variant in *FBN1* predisposes people to thoracic aortic disease in the absence of the skeletal and ocular features of Marfan syndrome.

Our GWAS data identified one locus associated with STAAD with GWS. This is in contrast to the several Mendelian genes known to predispose individuals to TAAD, in which substantial genetic heterogeneity is evident for both syndromic and nonsyndromic disease. Similarly, copy-number variant (CNV) analysis of STAAD has identified 47 CNVs enriched or unique in people with STAAD compared

with controls<sup>13</sup>. Both single-gene mutations and a recurrent CNV involving duplication of 16p13.1 are associated with specific thoracic aortic disease presentations<sup>14,15</sup>. The 15q21.1 locus is associated with all disease presentations except type B dissections, which probably allowed its identification in the discovery phase of this study. Future GWAS of thoracic aortic disease need to have subject cohorts of sufficient size to detect loci associated with specific subphenotypes.

In summary, we have identified novel associations of polymorphic variants at the 15q21.1 locus, encompassing *FBN1*, with STAAD. These data suggest that one or more common variants in *FBN1* predispose individuals to STAAD, implying a common pathogenesis of thoracic aortic disease between Marfan syndrome and STAAD. Our current understanding of the molecular pathways that lead to thoracic aortic disease is driven by studies of Marfan-syndrome mouse models. Blocking the signaling of transforming growth factor- $\beta$  with losartan attenuates aneurysm formation in the mouse models, and this observation has led to clinical trials of losartan to prevent aneurysm formation in people with Marfan syndrome<sup>16</sup>. In addition, inhibitors of matrix metalloproteinases and of the ERK signaling pathway have also shown efficacy in blocking aneurysm formation in mouse models of Marfan syndrome<sup>17,18</sup>. Finding a common pathologic pathway to thoracic aortic disease that is driven by genetic variants in *FBN1* (including mutations and common variants) may allow the effective treatments designed to slow or prevent thoracic aortic disease in patients with Marfan syndrome to be rapidly applied to patients with STAAD.

**URLs.** NINDS dbGAP database, <http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap>; WTCCC database, [https://www.wtccc.org.uk/cccl/access\\_to\\_data\\_samples.shtml](https://www.wtccc.org.uk/cccl/access_to_data_samples.shtml); R script from Broad Institute website, <http://www.broadinstitute.org/diabetes/scandinav/figures.html>.

## METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturegenetics/>.

*Note: Supplementary information is available on the Nature Genetics website.*

## ACKNOWLEDGMENTS

This work was supported by grants from the US National Institutes of Health (P50-HL083794 and R01-HL62594 to D.M.M.; UL1RR024148 and UL1RR025758 (CTSA); K08-HL080085 to S.A.L.), as well as the Doris Duke Charitable Trust, the Vivian L. Smith Foundation, the TexGen Foundation and the Thoracic Surgery Foundation for Research and Education.

GenTAC participating centers and their investigators are as follows: Johns Hopkins University School of Medicine, K.W.H., H.C. Dietz, W. Ravekes, K. Lurman; University of Texas Health Science Center at Houston, D.M.M., A. Carlson; Baylor College of Medicine, S.A.L., J.L. Jefferies, I.V. Volguina; Oregon Health & Science University, C.M., H.K. Song, V. Menashe, M. Silberbach, J.D. Kushner; Perelman School of Medicine at the University of Pennsylvania, R.E.P., J.E.B., M. Morales; Weill Cornell Medical College, C.T. Basson, R.D., J.W. Weinsaft, D. McDermott; University of Michigan Medical School, K.E.; US National Heart, Lung, and Blood Institute, H.E. Tolunay, P. Desvigne-Nickens, M.P. Stylianou, M. Mitchell; RTI International, B.L. Kroner, D. Brambilla, T. Hendershot, D. Ringer, M. Cunningham, M. Kindem. The GenTAC registry is supported by US Federal Government Contract N01-HV-68199 from the National Heart, Lung, and Blood Institute and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

We thank the GenTAC investigators, collaborators and coordinators; NINDS and WTCCC for giving us the use of their control data; S.N. Palmer, N. Stancel and R. Bartow for editorial assistance; Y. Khalil for subject recruitment; Z. Ren for technical support; A. Kicza for her dedicated work contacting and talking to subjects; and all of the subjects who participated in this research. We thank the surgeons of Brigham and Women's Hospital: S.F. Aranki, R.M. Bolman III, F.Y. Chen, L.H. Cohn, G.S. Couper, M. Davidson, R.J. Rizzo and P. Shekar. We also thank the surgeons of Massachusetts General Hospital: A. Agnihotri, C. Akins, A.D. Hilgenberg, T.E. MacGillivray, J.C. Madsen, B.R. Rosengard, D.F. Torchiana, G.J. Vlahakes and J.D. Walker.

## AUTHOR CONTRIBUTIONS

S.A.L., J.W.B. and D.M.M. were the principal investigators who conducted the study. S.A.L. and D.M.M. coordinated and oversaw the study. L.R., R.J.J., C.C.M., D.M.M., S.A.L., J.S.C., H.J.S. and A.L.E. participated in study enrollment for stages 1 and 2, helped gather related detailed clinical information and biological samples, and helped carry out the clinical analysis. R.E.P., J.E.B., R.D., C.M., K.W.H., K.E., S.C.B., C.S., J.G.S. and E.M.I. participated in study enrollment for stages 2 and 3 and helped gather related detailed clinical information and biological samples. D.G. and M.N. participated in the preparation of biological samples. M.B., J.W.B., M.R.B. and L.M.F. carried out the genotyping. M.-L.N.M. and S.M.L. were responsible for bioinformatics and all statistical analyses. S.A.L., M.-L.N.M., D.G., S.M.L. and D.M.M. wrote the manuscript.

## COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at <http://www.nature.com/naturegenetics/>.

Reprints and permissions information is available online at <http://www.nature.com/reprints/index.html>.

- Milewicz, D.M., Dietz, H.C. & Miller, D.C. Treatment of aortic disease in patients with Marfan syndrome. *Circulation* **111**, e150–e157 (2005).
- Guo, D.C. *et al.* Mutations in smooth muscle  $\alpha$ -actin (*ACTA2*) lead to thoracic aortic aneurysms and dissections. *Nat. Genet.* **39**, 1488–1493 (2007).
- Milewicz, D.M. *et al.* Genetic basis of thoracic aortic aneurysms and dissections: focus on smooth muscle cell contractile dysfunction. *Annu. Rev. Genomics Hum. Genet.* **9**, 283–302 (2008).
- Wang, L. *et al.* Mutations in myosin light chain kinase cause familial aortic dissections. *Am. J. Hum. Genet.* **87**, 701–707 (2010).
- Zhu, L. *et al.* Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. *Nat. Genet.* **38**, 343–349 (2006).
- Braverman, A.C. *et al.* The bicuspid aortic valve. *Curr. Probl. Cardiol.* **30**, 470–522 (2005).
- Svensson, L.G. Aortic valve stenosis and regurgitation: an overview of management. *J. Cardiovasc. Surg. (Torino)* **49**, 297–303 (2008).
- Svensson, L.G., Blackstone, E.H. & Cosgrove, D.M. III. Surgical options in young adults with aortic valve disease. *Curr. Probl. Cardiol.* **28**, 417–480 (2003).
- Larson, E.W. & Edwards, W.D. Risk factors for aortic dissection: a necropsy study of 161 cases. *Am. J. Cardiol.* **53**, 849–855 (1984).
- Hiratzka, L.F. *et al.* 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. *Circulation* **121**, e266–e369 (2010).
- Brautbar, A. *et al.* FBN1 mutations in patients with descending thoracic aortic dissections. *Am. J. Med. Genet. A.* **152A**, 413–416 (2010).
- Milewicz, D.M. *et al.* Fibrillin-1 (FBN1) mutations in patients with thoracic aortic aneurysms. *Circulation* **94**, 2708–2711 (1996).
- Prakash, S.K. *et al.* Rare copy number variants disrupt genes regulating vascular smooth muscle cell adhesion and contractility in sporadic thoracic aortic aneurysms and dissections. *Am. J. Hum. Genet.* **87**, 743–756 (2010).
- Kuang, S.Q. *et al.* Recurrent chromosome 16p13.1 duplications are a risk factor for aortic dissections. *PLoS Genet.* **7**, e1002118 (2011).
- Milewicz, D.M., Carlson, A.A. & Regalado, E.S. Genetic testing in aortic aneurysm disease: PRO. *Cardiol. Clin.* **28**, 191–197 (2010).
- Habashi, J.P. *et al.* Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science* **312**, 117–121 (2006).
- Holm, T.M. *et al.* Noncanonical TGF $\beta$  signaling contributes to aortic aneurysm progression in Marfan syndrome mice. *Science* **332**, 358–361 (2011).
- Xiong, W., Knispel, R.A., Dietz, H.C., Ramirez, F. & Baxter, B.T. Doxycycline delays aneurysm rupture in a mouse model of Marfan syndrome. *J. Vasc. Surg.* **47**, 166–172 (2008).

## ONLINE METHODS

**Study oversight.** The study protocols were approved by the institutional review boards at the University of Texas Health Science Center at Houston and Baylor College of Medicine, and all subjects provided written informed consent for study participation. Samples were also obtained from the GenTAC National Registry for the stage 2 study<sup>19</sup>. The GenTAC study protocols were approved by the institutional review boards at each of the five regional clinical centers, and all subjects provided written informed consent for study participation. The protocol for the stage 2 study was approved by the GenTAC Steering Committee. The protocol for the stage 3 study was approved by the Harvard Medical School Institutional Review Board. Stage 3 control samples were obtained from the BioServe Global BioRepository (BioServe Biotechnologies)<sup>20</sup>.

**DNA extraction.** Saliva, whole blood or buffy-coat samples were collected from each subject at enrollment and stored at  $-80\text{ }^{\circ}\text{C}$  until DNA extraction. The quantity of double-stranded DNA extracted was measured using PicoGreen (Invitrogen). Samples with a DNA concentration less than  $40\text{ }\mu\text{g/ml}$  were concentrated by using a SpeedVac system (Thermo Scientific); samples with a DNA concentration  $>150\text{ }\mu\text{g/ml}$  were normalized to  $100\text{ }\mu\text{g/ml}$ . The purity of the DNA samples was assessed using a NanoDrop spectrophotometer (Thermo Scientific).

**Subjects and controls.** A three-stage case-control design was used to analyze three sets of data from individuals of European descent. Stage 1 comprised a GWAS of 805 individuals with sporadic ascending aortic aneurysms or classic aortic dissection of the ascending or descending thoracic aorta (Stanford types A and B, respectively) who presented for treatment at the Texas Medical Center (TMC). The diagnosis of TAAD was confirmed by cross-sectional imaging in all subjects and by direct inspection during surgical repair in most subjects. The aorta was considered aneurysmal when its diameter exceeded 2 s.d. above normal diameter indexed to sex and body surface area<sup>10,21</sup>. The following exclusion criteria were used: age  $\leq 30$  years; aortic aneurysm associated with infection, aortitis or trauma; isolated pseudoaneurysm; diagnosis of TAAD with a known syndromic cause or a first-degree relative with TAAD; and transfusion within 72 h of blood collection. Of the 805 people in stage 1, 40 were excluded during data quality control, leaving 765 people in the final stage 1 cohort (Supplementary Table 1). The control group for stage 1 comprised 1,436 subjects whose data were obtained from the WTCCC C58 Birth Cohort and who were genotyped using the Illumina HumanHap 550K BeadChip array, as well as 1,009 controls whose data were obtained from the NINDS Repository's Neurologically Normal Control Collection and who were genotyped using the Human CNV370-Quad BeadChip array (dbGAP accession phs000004.v1.p1)<sup>22</sup>. The final control groups, whose members met quality-control criteria and were included in subsequent analyses, comprised 1,355 WTCCC C58 subjects and 874 NINDS subjects.

Stage 2 replication analyses were conducted on DNA samples from 406 individuals who met the inclusion criteria for stage 1. Quality-control procedures (see 'Data quality control', below) led to the removal of 21 subjects. The remaining cohort (Supplementary Table 1) included 240 people with STAAD who were treated at the TMC and 145 people with both congenital BAV and STAAD (documented by imaging studies or intraoperative findings) who were enrolled in GenTAC<sup>19</sup>. The stage 2 control group comprised 156 individuals without TAAD who were recruited in the TMC. After the application of quality-control criteria, the stage 2 control group comprised 150 people (39 healthy blood donors and 111 affected individuals in whom the absence of TAAD was confirmed by cross-sectional imaging). The stage 3 replication cohort consisted of DNA samples from 163 subjects enrolled at Harvard Medical School and 476 control subjects obtained from BioServe after quality control (Supplementary Table 1).

**Genotyping.** Genotyping was carried out using the BeadStation system (Illumina) and the Human CNV370-Quad BeadChip, and 327,687 markers out of the 370,000 SNPs were used for GWAS analysis. Briefly, genomic DNA was amplified and fragmented before being hybridized to the genotyping chip. A single-base pair extension reaction was then performed, followed

by staining with detectable fluorescent dye labels. The resulting products were imaged with the BeadArray Reader (Illumina). Allele detection and genotype calling were carried out using BeadStudio software (Illumina). Quality standards for staining, single-base extension, hybridization, stringency and nonspecific binding were verified. The average heterozygosity of SNPs on the Human CNV370-Quad BeadChip is 0.34 in the HapMap CEU (Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain (CEPH)). Samples from stage 2 subjects were genotyped for 2,409 SNPs using an InfiniumHD iSelect Custom Kit-STAAD\_REP (Illumina). These SNPs included 113 SNPs in the *FBNI* gene region selected on the basis of the stage 1 imputed and genotyped SNPs associated with STAAD with  $P < 1 \times 10^{-5}$  and 545 ancestry-informative markers (AIMs).

**Data quality control.** For the stage 1, 2 and 3 samples, data cleaning and analysis were carried out in PLINK<sup>23</sup>. Samples missing  $>2\%$  of their genotypes were removed because this can indicate a low-quality DNA sample, resulting in higher error rates for those markers that were genotyped. SNP markers were excluded if they were not successfully genotyped in more than 95% of the DNA samples or if the marker had a minor allele frequency of  $<1\%$ . In addition, SNPs were excluded if they significantly deviated from Hardy-Weinberg equilibrium ( $P < 1 \times 10^{-7}$ ) in controls. Remaining SNPs that had  $1 \times 10^{-3} \geq P > 1 \times 10^{-7}$  in controls were flagged for follow-up if they were associated with STAAD, and these SNPs were excluded when SNP marker loci were imputed. Furthermore, the reported sex of the individuals was verified by using markers on the X chromosome. Additionally, samples were removed if they showed excess homozygosity or heterozygosity ( $>4$  s.d. from the median). Finally, identity by state statistics was used to check for duplicates and to evaluate relatedness among samples.

To identify outliers, multidimensional scaling (MDS) was carried out on a subset of the GWAS genotype data that were in linkage equilibrium or in low levels of linkage disequilibrium in stage 1, and using the AIMs in stage 2 and 3. The data for the MDS analysis came from the four HapMap populations (CEU, YRI (Yoruba in Ibadan, Nigeria), JPT (Japanese in Tokyo, Japan) and CHB (Han Chinese in Beijing, China)) merged with either the stage 1 sample or the stage 2 or 3 samples. Samples that deviated by more than 3 s.d. from the median of MDS components 1 and 2 were removed in stage 1 (Supplementary Fig. 2a). In stages 2 and 3, assessment of the cluster plot indicated that the removal of samples that deviated by more than 3 s.d. from the median of MDS component 1 or 2 was appropriate (Supplementary Fig. 2b,c). To strengthen the evidence for associations with STAAD, we then tested for associations by using stage 1 cases and the NINDS controls and then using the stage 1 cases and WTCCC C58 controls. Initially, 2 SNPs (rs361147 and rs4705038) appeared highly associated with STAAD in stage 1; however, imputed stage 1 results and stage 2 replication results indicated that these were false positives, and these SNPs were excluded from further analysis (Supplementary Table 11).

**Association testing.** We performed logistic regression analyses for each SNP using an additive allelic effect that corresponds to a multiplicative model for the odds ratio. We verified that the additive model was the most appropriate for the findings in *FBNI* by testing a dominance parameter in the model, which proved to be not significant. In addition to analyzing the STAAD phenotype in stages 1, 2 and 3, we analyzed the following phenotype subsets by comparing each one to their respective stage 1, 2 and 3 controls: (i) STAAD with BAV, (ii) STAAD without BAV, (iii) nondissection ascending aortic aneurysm, (iv) aortic dissection, (v) type A dissection and (vi) type B dissection. For stage 3, aortic dissection and STAAD without BAV were not analyzed because of the low numbers of cases with those subphenotypes. We carried out genome-wide association testing on all stage 1 individuals with STAAD, comparing them first with the WTCCC C58 controls and then with the NINDS controls. Subphenotype analyses for stage 1 were carried out using the NINDS controls. To evaluate potential population substructure,  $\lambda$  (ratio of the median of the observed test statistics to the median test statistics under the null) was calculated before and after sex and MDS components were added as covariates to the model. MDS was used to control for population substructure. In stage 1, comparison of the subjects with STAAD and the WTCCC C58 controls

( $n = 302,055$  markers) showed evidence of population substructure in the sample ( $\lambda = 1.21$ ); however, adding one MDS component and sex decreased  $\lambda$  to 1.06. Comparing the subjects with STAAD and NINDS controls ( $n = 327,687$  markers) revealed only weak evidence of population substructure ( $\lambda = 1.03$ ), which was further reduced by the addition of one MDS component and sex ( $\lambda = 1.005$ ). Sex and the first component from the MDS analysis were included as covariates in the regression model for the stage 1 samples. In stage 2, initial assessment of  $\lambda$  revealed evidence of weak population substructure ( $\lambda = 1.10$ ), which was completely reduced by addition of the first two components generated from MDS of the AIMs ( $\lambda = 1$ ). In stage 3, the initial assessment of  $\lambda$  indicated no population substructure ( $\lambda = 1$ ), and only sex was used to adjust for confounding in subsequent analyses. After homogeneity between stage 1, 2 and 3 results was assessed using the Cochran Q test, a meta-analysis was carried out using the fixed-effects model ( $Q \geq 0.1$ ) and the random-effects model ( $Q < 0.1$ ).

**Imputation.** SNPs were imputed using MaCH software<sup>24,25</sup>. Phased haplotypes from HapMap CEU participants from stages 1 and 2 and from stage 3 were used separately to infer untyped markers<sup>26</sup>. Only those imputed markers with Rsq scores  $\geq 0.8$  were analyzed<sup>27</sup>. Instead of analyzing the most likely genotype call for each individual's imputed SNP, we analyzed genotype dosages from MaCH adjusting for sex and C1 from the stage 1 MDS analysis described above.

**Manhattan and regional association plots.** The R library gap was used to generate the Manhattan plot in **Figure 1**, and the script to generate the regional association plot was downloaded and modified from the Broad Institute website.

19. Eagle, K.A. Rationale and design of the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC). *Am. Heart J.* **157**, 319–326 (2009).
20. Mahan, S., Ardlie, K.G., Krenitsky, K.F., Walsh, G. & Clough, G. Collaborative design for automated DNA storage that allows for rapid, accurate, large-scale studies. *Assay Drug Dev. Technol.* **2**, 683–689 (2004).
21. Roman, M.J., Devereux, R.B., Kramer-Fox, R. & O'Loughlin, J. Two-dimensional echocardiographic aortic root dimensions in normal children and adults. *Am. J. Cardiol.* **64**, 507–512 (1989).
22. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678 (2007).
23. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
24. Li, Y. & Abecasis, G. Mach 1.0: rapid haplotype reconstruction and missing genotype inference. *Am. J. Hum. Genet.* **S79**, 2290 (2006).
25. Li, Y., Willer, C., Sanna, S. & Abecasis, G. Genotype imputation. *Annu. Rev. Genomics Hum. Genet.* **10**, 387–406 (2009).
26. International HapMap Consortium. A haplotype map of the human genome. *Nature* **437**, 1299–1320 (2005).
27. Li, Y., Willer, C.J., Ding, J., Scheet, P. & Abecasis, G.R. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet. Epidemiol.* **34**, 816–834 (2010).