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# Identification of a genetic variant associated with abdominal aortic aneurysms on chromosome 3p12.3 by genome wide association

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***Objective:*** **The goal of this project was to identify genetic variants associated with abdominal aortic aneurysms (AAAs).**
***Methods:*** **A genome wide association study was carried out using pooled DNA samples from 123 AAA cases and 112 controls matched for age, gender, and smoking history using Affymetrix 500K single nucleotide polymorphism (SNP) arrays (Affymetrix, Inc, Santa Clara, Calif). The difference in mean allele frequency between cases and controls was calculated for each SNP and used to identify candidate genomic regions. Association of candidate SNPs with AAA was confirmed by individual TaqMan genotype assays in a total of 2096 cases and controls that included an independent replication sample set.**
***Results:*** **A genome wide association study of AAA cases and controls identified a candidate AAA-associated haplotype on chromosome 3p12.3. By individual genotype analysis, four SNPs in this region were significantly associated with AAA in cases and controls from the original study population. One SNP in this region (rs7635818) was genotyped in a total of 502 cases and 736 controls from the original study population ($P = .017$) and 448 cases and 410 controls from an independent replication sample ($P = .013$; combined $P$ value = .0028; combined odds ratio [OR] = 1.33). An even stronger association with AAA was observed in a subset of smokers (391 cases, 241 controls, $P = .00041$, OR = 1.80), which represent the highest risk group for AAA. The AAA-associated haplotype is located ~200 kbp upstream of the CNTN3 gene transcription start site.**
***Conclusion:*** **This study identifies a region on chromosome 3 that is significantly associated with AAA in 2 distinct study populations. (J Vasc Surg 2009;49:1525-31.)**

***Clinical Relevance:*** **Genotype data can be used to identify individuals at increased genetic risk for AAA. Ultimately this genetic information may lead to improved diagnosis and better understanding of the pathophysiology of AAAs.**

Abdominal aortic aneurysms (AAAs) are a leading cause of death in the elderly, usually from aneurysm rupture.[1,2] Many patients with AAAs are undiagnosed. The lack of diagnosis increases their risk of death from AAA, since surgical treatments to repair aneurysms before they rupture are safe and effective. The most important risk factors for AAAs are old age, male gender, smoking, and family history of AAA. The underlying causes of aneurysm formation are not known. Investigations into the pathophysiology of AAA have focused on remodeling of the extracellular matrix and inflammation as important mechanisms in AAA formation.[3-8]

Family and epidemiologic studies demonstrate a substantial genetic risk for AAA.[4,9] AAA is a complex disease with risk influenced by an unknown number of genes that act in combination with environmental factors such as smoking. Identifying specific genetic factors that are associated with AAA would be extremely useful in developing improved methods to diagnose and treat the disease. While a number of candidate gene association studies that examined genes selected on the basis of their predicted role in AAA have been reported,[4,9] none of these studies have conclusively identified any AAA-associated variants. Previously, whole genome linkage analyses using family-based or affected-relative-pair methods identified two AAA-associated genetic loci designated as the AAA1 (on 19q13) and AAA2

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(4q31) susceptibility loci.[4,10,11] The specific AAA-associated genetic variants in these regions have not yet been identified.

Another approach to identifying genetic variants associated with complex disease is a genome wide association study (GWAS) in unrelated groups of cases and matched controls, preferably using large numbers of genetic markers. The genetic markers of choice for GWAS are single nucleotide polymorphisms (SNPs). Most SNPs are bi-allelic, stable in the genome, and easily assayed. Several million common SNPs have been identified. In a typical GWAS, the majority of SNP alleles that are assayed have no known functional effects (eg, they do not alter protein coding information). However, because genetic variants that are physically close in the genome will generally be inherited together (are in linkage disequilibrium, linkage disequilibrium [LD]), SNPs that are nearby a functional genetic variant can serve as markers for the functional variant. A previous GWAS identified a genetic variant on chromosome 9 that is associated with a moderate increase in AAA risk.[12]

We carried out a case-control GWAS using a DNA pooling strategy that identified a candidate AAA-associated genomic region on chromosome 3p12.3. An SNP within this region was significantly associated with AAA in two independent study populations.

## METHODS

### Study populations

**Geisinger vascular clinic (GVC) samples.** The primary case and control groups consisted of patients enrolled through the Geisinger Clinic Department of Vascular Surgery. Patients had a diagnosis of AAA (defined as an aortic diameter of 3 cm or greater) as determined by abdominal imaging study and confirmed by an attending vascular surgeon. Controls were patients of the same clinic that were AAA-free, based on evaluation by a vascular surgeon of an abdominal imaging study carried out within the preceding 5 years. At enrollment, all participants provided a blood sample for DNA extraction, completed a questionnaire that collected information about smoking history, family history of AAA, and other information potentially related to AAA risk factors. Subjects also gave permission to extract clinical and demographic information from their electronic medical record (EMR). Characteristics of the GVC subjects are provided in Supplementary Table I, online only.

**Geisinger MyCode samples.** A secondary control group was obtained through the Geisinger MyCode Project, a population cohort of Geisinger Clinic primary care patients recruited for genomic studies. Enrolled participants agreed to provide blood samples for genomic research and permission to extract clinical and demographic data from their EMR. For this study, DNA samples from 442 MyCode participants were randomly selected from a collection of DNA samples from more than 5000 patients and were matched for age distribution and gender to the GVC cases, and excluded patients with a diagnosis of AAA in their EMR.

**Replication sample set.** These samples consisted of 453 AAA cases and 418 controls that were recruited from Belgium, Canada, and the USA. These samples have been used in other genetic studies of AAA, and have been described previously.[12]

Appropriate institutional approvals were obtained for use of human subjects in this research.

**DNA purification.** DNA was purified from EDTA-anti-coagulated blood using a Qiagen BioRobot M48 Workstation and MagAttract DNA Blood Midi M48 kit (Qiagen, Valencia, Calif). The yield and purity of DNA was determined by measuring the absorbance at 260 and 280 nm using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, Mass).

**Genome wide association study.** We carried out an initial GWAS of AAA cases and controls using a DNA pooling strategy similar to that used in previous studies.[13-16] A flow-chart that summarizes the GWAS strategy is in Supplemental Fig 1, online only. Three identical DNA pools were generated independently from DNA samples of 123 cases or 112 controls (a total of 6 pools) from the GVC samples. Cases and controls were matched for male/female ratio and age; all had a smoking history of >20 years. The characteristics of the subjects used for the GWAS are shown in Supplementary Table II, online only. To create the DNA pools, 100 ng of DNA from each subject was combined and then precipitated at −20°C by adding 0.1 volume of 3 M sodium acetate, pH 5.2, and 2.5 volumes of ethanol. The DNA was collected by centrifugation, washed with 70% ethanol, and air-dried. The dried pellets were dissolved in water at a final concentration of 50 ng/mL.

The six DNA pools were hybridized separately to Affymetrix SNP arrays using the GeneChip Mapping 500K Manual Protocol (Affymetrix, Inc, Santa Clara, Calif). Pooled DNA (250 ng/array) was digested with Nsp I or Sty I and ligated to adapters that recognize overhangs generated by the restriction enzymes. Oligonucleotides that recognize the adapter sequences were used as primers to amplify the adapter-ligated DNA fragments. The amplified DNA was fragmented, labeled, and hybridized to GeneChip Human Mapping 250K Nsp and 250K Sty arrays (Affymetrix, Inc) for 18 hours at 47°C. After hybridization, the chips were washed and stained using an FS-450 mapping 500Kv1-450 fluidics workstation (Affymetrix, Inc). The arrays were scanned using the GeneChip Scanner Model 3000 7G. The raw data were analyzed using the Affymetrix GeneChip Operating Software (GCOS), version 1.4.0.036, and GeneChip Genotyping Analysis Software, version 4.1.026 (GTYPE).

The relative frequencies of the two alleles for each SNP were determined from the hybridization signals. Only the perfect match probe signals were used in the allele frequency calculation. To identify SNPs with a high measurement variance, the intra-group coefficient of variation (CV) of the allele frequency for each SNP was calculated. SNPs with a CV ≥0.1 in either the case or control group were discarded. For the remaining 306,330 SNPs, the difference in mean allele frequency between cases and controls was

calculated. The *t* test *P* values for the allele frequency difference between cases and controls for each SNP were also calculated.

Candidate SNPs for follow-up studies were chosen from genomic regions that contained multiple SNPs with highest case-control allele frequency differences. This was based on the assumption that the high density of SNPs on the arrays (median distance between SNPs on the arrays is 2.5 kb) would reveal multiple SNPs in LD with the genetic variants contributing to the disease. To identify such regions, the SNP allele frequency difference data were sorted by physical position on each autosomal chromosome. Rolling sums of the relative allele frequency differences of five adjacent SNPs or sums of the –log10 transformed *P* values of the SNP allele frequency differences of five adjacent SNPs along each chromosome were calculated.

**Individual genotype analysis.** Individual genotype assays were performed using the Applied Biosystems (Foster City, Calif) 7500 Fast Real-Time polymerase chain reaction (PCR) System and the standard protocol for the MicroAmp Fast Optical 96-well reaction plate format (Affymetrix, Inc). PCR amplification was carried out in a 10 μL reaction using 10 ng of genomic DNA, TaqMan Genotyping Master Mix, TaqMan SNP Genotyping Assay probes (Applied Biosystems), and DNase-free water. Assay conditions were as follows: pre-read hold at 60°C for 30 seconds, fluorescence recording; 1 cycle at 95°C for 10 minutes to activate AmpliTaq Gold DNA Polymerase (Affymetrix, Inc); 40 cycles of 95°C for 15 seconds and 60°C for 1 minute followed by a fluorescence recording after each cycle; and a hold at 60°C for 30 seconds followed by an endpoint fluorescence recording. Data were analyzed using the 7500 Fast Sequence Detection System Software version 1.4 (Applied Biosystems), with background fluorescence subtraction and automatic allele calling. For SNP rs7635818, the genotype call rates were 99.9% with the Geisinger Clinic DNA samples and 98.8% with the replication samples.

**Contactin-3 transcript assay.** Aortic tissue was homogenized in Trizol reagent (Invitrogen, Carlsbad, Calif) and total ribonucleic acid (RNA) was extracted following the standard protocol. Mononuclear cells were obtained from blood samples collected in Cell Preparation Tubes with Citrate (BD Diagnostics, Franklin Lakes, NJ). RNA was isolated using QIAshredder spin columns and RNeasy kits (Qiagen, Valencia, Calif). Yield and quality of RNA were determined by measuring the absorbance at 260 nm and 280 nm.

Total RNA was used as a template for cDNA synthesis in a reaction that contained the following: 2 μg RNA, 4 μl of 10X Mg free buffer (Promega, Madison, Wis), 5 mM $MgCl_2$(Promega, Madison, Wis), 5 μM random hexamers, 1 mM dNTPs, 2 μl Superscript II RT (Invitrogen), and PCR grade water to a final volume of 40 μl. Reaction conditions were 25°C for 10 minutes, 50°C for 50 minutes, 70°C for 15 minutes followed by a 4°C hold.

cDNAs were used as templates for PCR amplification of *CNTN3* transcripts using JumpStart Taq DNA Polymerase (Sigma, St Louis, Mo). The standard protocol was used, but with the addition of $MgCl_2$ to a final concentration of 15 mM. Primers for the *CNTN3* gene were designed using PrimerQuest (Integrated DNA Technologies, Coralville, Iowa). Primer pairs used were: forward 5' – TGCCCTTGGAAATCCCATACCTCA – 3' and reverse 5' – TGTCCTCCACGGCTATTTCCACAT – 3' (predicted product size of 251 bp); and forward 5' – ACAGACACAACAGCCCAACTCTCT – 3' and reverse 5' – GGCGGAAAGCAACAACATACCCAA – 3' (predicted product size of 397 bp). PCR amplification conditions were 1 minute at 94°C followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes and a 4°C hold. Products were analyzed on 1% agarose gels and stained with ethidium bromide.

**Statistical analyses.** Genotype data were analyzed using Helix Tree Software, version 6.4 (Golden Helix, Inc, Bozeman, Mont). Analyses included calculations of deviation from Hardy-Weinberg equilibrium, linkage disequilibrium, and genetic association. *P* values for genetic association were calculated using recursive partitioning with case/control selected as a categorical dependent variable and genotype as the independent variable. The data were also analyzed assuming a dominant model of genetic association, which tests the association of having at least one minor allele vs having no copies of the minor allele. Both approaches yielded essentially identical results. Uncorrected *P* values are reported.

Power calculations were performed using the Genetic Power Calculator.[17] We assumed that the polymorphism and the disease locus were in complete LD and had the same allele frequencies. Power was calculated for different model parameters. Assuming a disease prevalence of 0.05 and risk allele frequency of 0.4, the Geisinger Clinic sample size of 502 cases and 748 controls had an 80% power to detect a susceptibility locus with a genotypic relative risk (GRR) of 1.42 for a dominant genetic model at a significance level of 0.1. The combined data set of 995 cases and 1154 controls could detect a dominant disease locus with a GRR of 1.34 with 80% power at a significance level of 0.05.

## RESULTS

**GWAS identifies a variant on 3p12.3 associated with AAA.** As the first part of a multi-stage approach to identify AAA-associated genetic variants, we carried out a GWAS using a subset of GVC case and control DNA samples. Relative SNP allele frequencies in triplicate pools of 123 case and 112 control samples were determined using Affymetrix 500K SNP arrays. (See Supplementary Fig 1, online only, for additional details on the GWAS.) Criteria for prioritizing candidate SNPs included a combination of absolute case-control SNP allele frequency differences, *t* test *P* values of SNP allele frequency differences, and physical clustering of SNPs with allele frequency differences.

Twenty genomic regions on 13 autosomal chromosomes that contained clusters of SNPs with significant allele frequency differences between the case and control groups

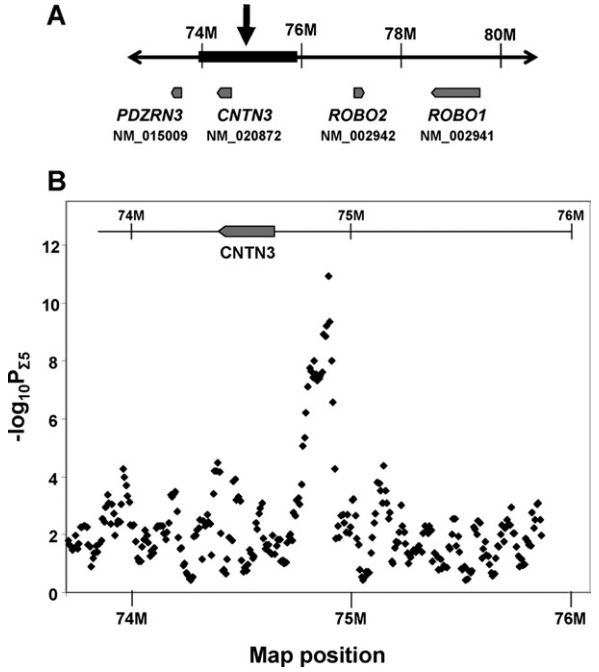
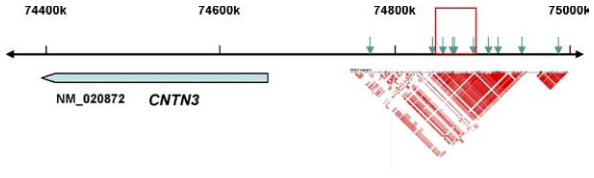



**Fig 1.** Genome wide association identifies a candidate abdominal aortic aneurysm (AAA)-associated region on chromosome 3p12.3. **A,** The *vertical arrow* indicates a region on chromosome 3 that contains SNPs with significant allele frequency differences between AAA cases and controls. The known genes in this region are indicated below the line with their gene names and accession numbers. Vertical lines and numbers above the line indicate chromosomal position (in millions of base pairs). **B,** Transformed *P* values of case-control allele frequency differences corresponding to the region indicated by the *thick vertical bar* in panel **A** are shown. Each point represents the sum of 5 adjacent $-\log_{10} P$ values. The physical map is shown for reference. Note the peak of $-\log_{10} P$ value sums located ~200,000 base pairs upstream of the *CNTN3* gene.




| NCBI SNP Reference | HapMap Position | P-value |
|---|---|---|
| rs6549634 | 74762904 | 0.191 |
| rs12714811 | 74841440 | 0.300 |
| rs7642555 | 74852110 | 0.039 |
| rs7635639 | 74866049 | 0.007 |
| rs7635818 | 74866166 | 0.004 |
| rs1569066 | 74881275 | 0.044 |
| rs10222550 | 74902382 | 0.120 |
| rs1527527 | 74919636 | 0.153 |
| rs2178032 | 74943083 | 0.120 |
| rs4417905 | 74998753 | 0.547 |

**Fig 2.** Genotype analysis and haplotype structure of the abdominal aortic aneurysm (AAA)-associated region on chromosome 3p12.3. Vertical *blue arrows* indicate the physical positions of the 10 SNPs from this region that were individually genotyped in 451 cases and 279 controls from the Geisinger Vascular Clinic (GVC) sample set. The position of the *CNTN3* gene is shown. The *inverted red triangle* is the HapMap $R^2$ linkage disequilibrium (LD) plot for the Caucasians of European descent from Utah (CEU) population (*red* indicates highest $R^2$ values). The table shows the reference numbers, map positions, and $\chi^2$ *P* values for AAA association of the genotyped single nucleotide polymorphisms (SNPs). The significantly associated SNPs are outlined by *red boxes* and lie within a CEU haplotype block characterized by strong LD.

(Fig 1) were selected for initial analysis by SNP genotype assay in a sample consisting of 550 GVC cases and controls. A region on chromosome 3p12.3 showed the strongest association with AAA, and was selected for further analysis, as described in the following sections.

**Validation of results through individual SNP genotyping.** To conditionally validate the association of this genomic region with AAA, 10 SNPs in this region were genotyped in a total of 502 GVC cases and 296 control samples. Four SNPs, including rs7635818, were significantly associated with AAA (Fig 2). The AAA-associated SNPs are contained within a haplotype block in the HapMap CEU (Caucasians of European descent from Utah) population (Fig 2) and were in strong LD in the GVC case and control samples (D' ≥0.98) (Supplementary Fig 2, online only). SNP rs7635818 was genotyped in an additional set of 442 controls obtained from the Geisinger MyCode population that were matched to the GVC case samples for age and gender. In the combined Geisinger samples, (502 cases and 748 controls) SNP rs7635818 genotype was significantly associated with AAA ($P = .017$) (Table). The AAA-associated allele was C (with G as the major allele) and its frequency was 0.48 in cases and 0.42 in controls.

**Table.** Association of SNP rs7635818 with AAA disease

| | | *Genotype frequency* | | | | |
|---|---|---|---|---|---|---|
| | | *CC/CG* | | *GG* | | P |
| *Sample set* | *N* | *Case* | *Control* | *Case* | *Control* | *value* |
| Geisinger Clinic | 502 cases<br>736 controls | .73 | .67 | .27 | .33 | .017 |
| Replication set | 448 cases<br>410 controls | .73 | .67 | .27 | .33 | .013 |
| All samples | 950 cases<br>1146 controls | .73 | .67 | .27 | .34 | .0028 |

*SNP,* Single nucleotide polymorphism; *AAA,* abdominal aortic aneurysm; *N,* number.

**Replication of association in an independent sample set.** To further substantiate the genetic association with AAA, SNP rs7635818 was genotyped in an independent set of 453 case and 416 control samples obtained from Belgium, Canada, and the USA. SNP rs7635818 was significantly associated with AAA ($P = .013$) in this replication sample (Table I), providing additional evidence that this

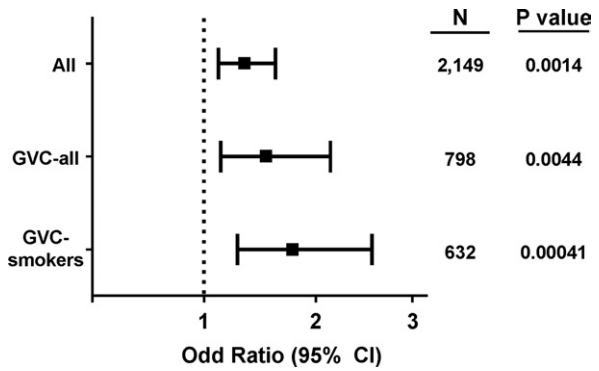



**Fig 3.** Odds ratios and *P* values for abdominal aortic aneurysm (AAA) association of single nucleotide polymorphism (SNP) s7635818. The graph shows the calculated odds rations (with 95% confidence intervals [CI]) for all samples combined, the set of all Geisinger Vascular Clinic (GVC) samples, and the GVC samples from subjects with 20 or more years of smoking history. The total sample size and $\chi^2$ *P* values are also shown. These values were calculated using Helix Tree Software (version 6.4; Golden Helix, Bozeman, Mont), assuming a dominant genetic model. The *dashed vertical line* indicates an odds ratio of 1.

region contains AAA-associated genetic variants. When the data were analyzed in all samples combined (955 cases and 1154 controls), SNP rs7635818 genotype was significantly associated with AAA ($P = .0028$) (Table I). A dominant genetic model yielded the most significant association between the SNP genotype and AAA.

**Interaction with smoking.** Smoking is a strong environmental risk factor for AAA disease. Because we had detailed smoking history data on the GVC case and control samples, we calculated the association of SNP rs7635818 with AAA in the subset of 391 cases and 241 controls with a smoking history of ≥20 years, and compared these data to those for the unstratified GVC cases and controls and the combined set of all cases and controls. In the combined sample set, the odds ratio (OR) for association of SNP rs7635818 with AAA was 1.33 (95% confidence interval [CI] 1.10-1.61, $P = .0014$) (Fig 3). In the unstratified GVC samples, the OR was 1.56 (95% CI 1.15-2.13, $P = .0044$), and in the GVC smokers-only samples the OR was 1.80 (95% CI 1.3-2.5, $P = .00041$). Thus, the association of SNP rs7635818 with AAA appears to be stronger in individuals with a history of smoking.

***CNTN3* is expressed in AAA and other vascular tissues.** SNP rs7635818 is upstream of the *CNTN3* gene, which encodes a lipid-anchored cell adhesion protein (contactin-3).[18] The *CNTN3* gene has not been reported previously to be expressed in vascular tissue. To assay *CNTN3* transcripts, RNA extracted from AAA tissue, control aortic tissue, aortic-occlusive disease (AOD) tissue, and peripheral blood mononuclear cells from AAA patients was used to generate cDNAs that were used as templates for PCR amplification using *CNTN3*-specific primers. Products derived from *CNTN3* transcripts were detected in all the tissues sampled (Fig 4), demonstrating

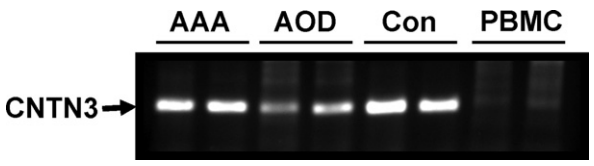



**Fig 4.** Expression of contactin-3 transcripts in aortic tissue. Total ribonucleic acid (RNA) isolated from abdominal aortic aneurysm (AAA) tissue, control (normal) aortic tissue, aortic occlusive disease tissue (AOD), or peripheral blood mononuclear cells (PBMC) from patients with AAA were used as templates for reverse transcriptase and polymerase chain reaction (PCR) amplification using contactin-3 specific primers. The products were resolved on an agarose gel and stained with ethidium bromide. The expected position of the contactin-3-specific product is indicated by the *arrow*. Two independent samples of each type were analyzed. Identical results were obtained with a different pair of contactin-3-specific PCR primers (data not shown). *Con,* xxx.

that the gene is actively expressed in aortic tissue, including AAA specimens.

## DISCUSSION

We carried out a genome wide association study to identify genomic variants associated with AAA. A DNA pooling strategy in conjunction with high density allele frequency determinations using Affymetrix 500K SNP arrays identified a candidate genomic region on chromosome 3p12.3. To validate the association of this region with AAA, several SNPs in this region were individually genotyped in additional case and control samples. Genetic association was also confirmed in an independent replication sample set. One SNP in this region, rs7635818, was genotyped in a total of 2096 case and control samples. SNP rs7635818 genotype was significantly associated with AAA, with a $\chi^2$ *P* value of .0028 and a calculated OR of 1.33 (1.10-1.61 95% CI). This is comparable to the OR of the previously reported AAA-associated SNP on chromosome 9.[10]

The Affymetrix chips we used contained probes for approximately 500,000 common SNPs, which are located throughout the genome; most do not overlap known genes. These differ from other gene chips that are used to measure gene activity or expression. An advantage of a genome wide association study of the type reported here is that the outcome is not dependent on a priori assumptions about the underlying biological mechanisms.

SNP rs7635818 is probably not the genetic variant that is functionally responsible for increased AAA risk. Rather, it is likely that this SNP serves as a marker for a functional genetic variant that increases AAA risk that is in LD with the marker SNP. SNP rs7635818 is contained within a haplotype block that shows strong LD in the HapMap CEU population and in the case and control groups used for this study. Our genetic mapping studies showed the AAA-associated haplotype block that contains SNP rs7635818 to span approximately 30 kb of genomic DNA sequence. This region does not overlap any known protein coding

genes, but is approximately 200 kb upstream of the transcription start site for the *CNTN3* gene. (The next nearest genes are PDZRN3 and ROBO2, which are approximately 1.3 and 2.2 million bp away, respectively.) There are no non-synonymous SNPs in *CNTN3* reported in public databases that change the predicted structure of activity of the *CNTN3* gene product. Together, these results suggest that the functional genetic variant has a regulatory function. Because it lies upstream of the *CNTN3* gene, where sequence motifs that regulate *CNTN3* gene activity are expected to be located, our working hypothesis is that the functional variant is in the upstream regulatory region of the *CNTN3* gene. The product of the *CNTN3* gene is a lipid-anchored cell adhesion protein that had been previously shown to be expressed in the central nervous system.[18] In support of this hypothesis, we found that the *CNTN3* transcript is readily detected in aortic tissue, including both normal and AAA tissue. Although this genomic region contains consensus DNA binding sites for known transcription factors, no studies to date have examined the effects of sequence variants in the AAA-associated haplotype block marked by SNP rs7635818 on *CNTN3* expression. Unfortunately, the limited number of high quality tissue samples that were available from AAA and risk-matched normal aortas did not allow a quantitative analysis of contactin-3 mRNA levels to determine whether the genetic variant altered expression of the gene. Moreover, since the cellular and biochemical changes that initiate AAA formation, the events most likely to be influenced by genes that increase AAA risk, occur well before the lesions are diagnosed clinically and repaired, it is not feasible to obtain biological samples from humans to test this hypothesis directly.

A dominant genetic model yielded the most significant association between SNP rs7635818 genotype and AAA. This would be consistent with the existence of a genetic variant in the *CNTN3* promoter that affects contactin-3 expression, either constitutively or in response to regulatory signals. Regulation of contactin-3 expression in vascular tissue has not been investigated.

A potentially important suggestive finding from the study was that the genetic risk associated with SNP rs7635818 was greater in individuals with a history of smoking. Smoking is a strong non-genetic risk factor for AAA. These findings suggest a gene-environment interaction that influences AAA risk. Because smoking history data were only available on the subjects recruited from the Geisinger Vascular Clinic, there were not sufficient numbers of non-smoking cases or controls to carry out an independent calculation of genetic risk in non-smokers. It is also interesting to note that the initial GWAS was carried out using only cases and controls with a history of smoking. This might explain why a relatively underpowered GWAS identified this genomic region. The matching for smoking history in the GWAS and demonstration of genetic association in the smoking sub-group also rules out the possibility that the observed association is related to smoking or a related characteristic (eg, smoking addiction).

These considerations also highlight the value of DNA samples from thoroughly characterized subjects for genetic association studies. A sufficient breadth of data allows for careful matching of cases and controls for known risk factors and the ability to examine interactions among genetic and non-genetic risk factors. It is also important to note that the GVC controls were screened to eliminate patients with undiagnosed AAA, which has been estimated to occur in up to 6% of individuals in the highest risk group, ie, male smokers over the age of 70 years. This could explain why the *P* value for association of SNP rs7635818 with AAA was not as robust in the replication sample set compared to the Geisinger samples, as these were not as closely matched for the other risk factors (eg, male gender) and were not screened for undiagnosed AAA.

The genetic variant reported here has a moderate but statistically significant association with AAA disease. This variant and the previously reported SNP on chromosome 9p21 account for only a portion of the genetic risk for AAA. Studies are ongoing to discover additional AAA-associated genetic variants. A long-term goal of this research is to use genetic information to quantify AAA disease risk, probably through the use of a panel of AAA-associated genetic variants. This would enable high-risk individuals to be identified for closer monitoring to detect undiagnosed AAAs, so that appropriate treatment can be provided in a timely manner. Knowledge of disease-associated genetic variants can also provide new insight into disease mechanisms and provide targets for development of novel therapies.[19] At the present time, there are no medical treatments for AAAs. The proximity of this AAA-associated genetic variant to the *CNTN3* gene, for example, should provide the impetus to study that gene and its product, contactin-3, in the context of vascular disease.

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## AUTHOR CONTRIBUTIONS

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Critical revision of the article: JE, MO, HK, GT, GG, DF, AB, DC
Final approval of the article: JE, MO, HK, GT, GG, DF, AB, DC
Statistical analysis: HK, GT, DC
Obtained funding: JE, HK, GT, GG, DC
Overall responsibility: DC

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