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Association of TGFB2 Polymorphism with Risk of Sudden Cardiac Arrest in Patients with Coronary Artery Disease

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Abstract

Background: Transforming growth factor β (TGF β) signaling has been shown to promote myocardial fibrosis and remodeling with coronary artery disease (CAD), and previous studies demonstrate a major role for fibrosis in the initiation of malignant ventricular arrhythmias (VA) and sudden cardiac arrest (SCA). Common single nucleotide polymorphisms (SNPs) in TGF β pathway genes may be associated with SCA.

Objective: We examined the association of common SNPs among 12 candidate genes in the TGF β pathway with the risk of SCA.

Methods: SNPs (n=617) were genotyped in a case-control study comparing 89 patients with CAD and SCA due to VA to 520 healthy controls.

Results: Nineteen SNPs among 5 genes (TGFB2, TGFB2, SMAD1, SMAD3, SMAD6) were associated with SCA after adjustment for age and sex. After permutation analysis to account for multiple testing, a single SNP in TGFB2 (rs9838682) was associated with SCA (OR = 1.66, 95% CI = 1.08-2.54, p=0.02).

Conclusion: We demonstrate an association between a common TGFB2 polymorphism and risk of SCA due to VA in the setting of CAD. If validated, these findings support the role of genetic variation in TGF β signaling in SCA susceptibility.

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Keywords

Sudden cardiac arrest; ventricular tachycardia; ventricular fibrillation; coronary artery disease; transforming growth factor β ; genetics

Introduction

Sudden cardiac arrest (SCA) remains a major public health problem, accounting for up to 450,000 deaths per year in the U.S.¹ Previous studies suggest that approximately 80% of sudden cardiac deaths (SCD) occur in the setting of coronary artery disease (CAD).² Ventricular tachycardia (VT) or ventricular fibrillation (VF) is the initiating event in the majority of SCA cases.³ Ejection fraction (EF) remains the only major criterion to stratify patients for risk of SCD and implantable cardioverterdefibrillator (ICD) implantation, but this strategy alone is insensitive and nonspecific.⁴ Genetic susceptibility to SCA in the setting of CAD is supported by several epidemiologic studies demonstrating that a family history of SCD is an independent risk factor for SCD and primary VF.^{5, 6, 7}

Transforming growth factor β (TGF β) is a potent profibrotic cytokine. Three structurally similar isoforms (TGF- β 1, β 2, and β 3) encoded by three distinct genes transduce their signal through combinations of transmembrane type I and type II receptors (TGFBR1 and TGFBR2) and their downstream effectors, the SMAD proteins.^{8, 9} TGF β is expressed at high levels in the heart during embryonic development and adult life, localizing in both cardiomyocytes and the extracellular matrix.¹⁰ TGF β 2 knockout mice exhibit perinatal mortality and a wide range of developmental, including cardiac, defects.¹¹ Regulatory mutations in the TGF β 3 gene cause arrhythmogenic right ventricular cardiomyopathy, an autosomal dominant clinical syndrome of ventricular arrhythmias, SCD, and fibrofatty replacement of the right ventricle.¹² Members of the TGF β family are markedly induced in infarcted myocardium and play a central role in infarct healing, cardiac repair, and left ventricular remodeling through mechanisms of cardiomyocyte growth, fibroblast activation, and extracellular matrix deposition.¹³ Although these basic and genetic studies suggest a central role for the TGF β signaling pathway in ischemic remodeling, genetic variation in members of the pathway have not been investigated for association with risk of ventricular arrhythmias and SCA in the setting of CAD.

We examined the potential role of polymorphisms in 12 genes in the TGF β signaling pathway in a group of patients with a history of myocardial infarction (MI) and aborted SCD with documented VT/VF compared to three healthy control groups without SCA or ventricular arrhythmias.

Methods

The UCSF Committee on Human Research approved all protocols. Informed consent was obtained from all participants for DNA isolation and plasma collection.

Study Design—Consecutive cases of SCA presenting to UCSF Medical Center Emergency Department or inpatient cases of SCA at UCSF Medical Center between January 2000 and June 2008 were screened. SCA was defined as a cardiac arrest with documented sustained monomorphic VT or VF requiring cardioversion or defibrillation, exclusive of torsades de pointes (drug-induced QT prolongation or otherwise). Because we were interested in the most common phenotype of SCA, that occurring in the setting of CAD, only patients with a history of MI were included in this study. Although all SCA cases had a history of MI, thirty-six SCA cases occurred in the setting of acute ischemia while 53 SCA cases occurred in the absence of

active ischemia. Thus, SCA cases consisted of 89 Caucasian non-Hispanic patients with aborted SCD and a history of MI.

The control population originated from three resources.¹⁴ These were randomly selected, healthy Caucasian controls without specific information regarding CAD. Recent large genome-wide association studies using shared controls of this type have met with success.¹⁴⁻¹⁶ Sixty unrelated controls employed by the International HapMap Project (<http://www.HapMap.org>) were selected from the Coriell Institute for Medical Research (<http://ccr.coriell.org>) Human Genetic Data Collection (sample identifiers are available on request). 216 healthy Caucasian controls were derived from a large genome wide association study of narcolepsy.¹⁴ The remaining 244 controls were healthy Caucasian renal transplant donors from an ongoing study of the genomics of renal transplantation (NIH U19 AI063603). Informed consent was obtained from all participants. Protocols were approved by the local institution review boards at all participating institutions.

Gene Selection: Figure 1 describes the TGF β -related signaling pathway. The SMAD mediators can be divided into two main intracellular pathways: SMAD2/3 or SMAD1/5.¹⁷ Members of the TGF β family (TGF β 1/2/3) bind TGFBR2, Type 2 receptor, a specific serine/threonine receptor kinase which catalyzes the phosphorylation of Type 1 receptor, TGFBR1, activating the protein. TGF β Type 3 receptor, TGFBR3, may enhance the binding of TGF β ligands to TGFBR2 by binding TGF β and presenting it to TGFBR2. Activated type I receptors phosphorylate specific receptor regulated R-SMADs, the intracellular effectors of TGF β signaling, either SMAD2/3 or SMAD 1/5. Activated RSMADs form heteromeric complexes with SMAD4 that translocate to the nucleus, where they regulate the expression of target genes in conjunction with transcription factors. Inhibitory SMADs, SMAD6 and SMAD7, antagonize TGF β signaling by inhibiting activation of R-SMADs. We examined twelve genes (TGFB2, TGFB3, TGFBR1, TGFBR2, TGFBR3, SMAD1, SMAD2, SMAD3, SMAD4, SMAD5, SMAD6, SMAD7) in the TGF β signaling pathway to examine for association with risk of SCA. TGFBR1 was not included since the only variant on the array (see below) did not meet our criteria for a common variant (allele frequency of <0.05) and thus was not analyzed.

Blood collection and genotyping—Blood samples were obtained by venipuncture and genomic DNA was extracted from peripheral blood lymphocytes (Invitrogen, Carlsbad, CA) for the SCA cases. Genotyping was performed blinded to clinical status; positive and negative controls were included. DNA samples were quantitated with a Nanodrop Spectrophotometer (ND-1000) and normalized to a concentration of 50 ng/ μ L (diluted in 10 mM Tris/1 mM EDTA). Samples were genotyped using the Genome-Wide SNP Array 6.0 (Affymetrix, Santa Clara, CA) and processed according to the standard Affymetrix automated protocol. This involved fragmentation, whole genome amplification, precipitation, resuspension in hybridization buffer and hybridization to the Genome-Wide SNP Array 6.0. After hybridization the arrays were processed and imaged on an Affymetrix GeneChip® Scanner. Genotype data for the controls were obtained using the same protocols in the same laboratory as described for SCA cases.

SNP Selection—Common variants (defined as having a minor allele frequency ≥ 0.05 in the combined sample of cases and controls) mapping to the candidate genes of interest were extracted from the array .cel files. Data files (Affymetrix “.cel” files) were obtained for all samples (n=608) and genotype calls were assigned using the Birdseed-dev algorithm for Affy 6.0 (Affymetrix Power Tools apt-1.8.5). In order to ensure robust genetic association analyses, we performed quality control filtering of both samples and SNPs. For each Birdseed calling run, SNPs with call rates <95%, or Hardy-Weinberg $p < 0.001$ in controls were excluded. A total of 617 SNPs among the 12 candidate genes (SMAD1: 25 SNPs; SMAD2: 22 SNPs; SMAD3: 65 SNPs; SMAD4: 7 SNPs; SMAD5: 18 SNPs; SMAD6: 147 SNPs; SMAD7: 21

SNPs; TGFB2: 47 SNPs; TGFB3: 7 SNPs; TGFBR1: 24 SNPs; TGFBR2: 125 SNPs; TGFBR3: 109 SNPs) passed all quality control filters and were included in the genetic association analyses. SNP selection was performed using the HelixTree SNP & Variation Suite 7 (GoldenHelix, Bozeman, MT). Potential functional roles of SNPs associated with SCA were examined using PUPASuite 2.0,¹⁸ a comprehensive search engine that tests a series of functional effects (i.e., non-synonymous changes, altered transcription factor binding sites, exonic splicing enhancing or silencing, splice site alterations, microRNA target alterations).

Statistical Analyses

To examine for differences in the distribution of demographic factors between cases and controls, we used the unequal-variance t-test for age and a chi-square test for sex. These analyses were carried out using STATA 9.0.

Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the chi-square exact test. Measures of linkage disequilibrium, D' and r^2 , were computed from the case and control genotypes with Haploview 4.1 (<http://www.broad.mit.edu/mpg/haploview/>) and heat maps of pairwise D' values are presented in Supplemental Figures 1-11. Homogeneity in ancestry between cases and shared controls was verified by cluster and principal component analysis (PCA).¹⁹ To investigate other biases that could be introduced by using controls shared with other studies such as batch effects due to differences in instrumentation,²⁰ we assessed the potential effect of substructure with the genomic-control method²¹ and with PCA, as implemented in HelixTree (GoldenHelix, Bozeman, MT). Briefly, the number of principal components were sought which minimized the value of the genome control test statistic. Population substructure was found to be negligible, with no discernible batch effects and PCA-adjusted tests of association that varied little as compared with unadjusted tests (data not shown).

For association tests, four genetic models were assessed for each SNP: dominant, recessive, log additive, and codominant. Barring trivial improvements ($\Delta < 10\%$) the genetic model that best fit the data, by maximizing the significance of the p-value was selected for each SNP. Both un-adjusted and adjusted associations were calculated; logistic regression was used to control for age and sex. Genetic model fit and both unadjusted and age- and sex-adjusted odds ratios were estimated using the SAS software package, version 9.1.3 (SAS Inc, Cary, NC).

Permutation tests were used to protect the type-I error rate against inflation due to testing of multiple SNPs within each gene. To account for multiple comparisons, case-control status was permuted 10,000 times to determine the likelihood that our findings were due to chance. Permuted significance tests are presented in Supplemental Tables 1-12. Permutation analyses were done using the PLINK software package (v1.06).²²

Results

Study Population

Compared with controls (Table 1), SCA cases were older (73.0 ± 12.7 versus 52.7 ± 15.6 years; $p < 0.0001$) and predominantly male (90.9% versus 51.9%, $p < 0.0001$). SCA cases had a mean BMI of 25.7 ± 4.4 kg/m² and a mean EF of $35.7 \pm 14.5\%$. History of congestive heart failure (CHF) was present in 76.5% of cases; diabetes mellitus was present in 28.4% of cases, and hypertension in 81.6% of cases. Clinical characteristics were not available for the anonymous healthy controls.

Genetic Association of TGFBR2 rs9838682 with SCA

Genotype frequencies for each SNP for each of the 12 candidate genes are shown in Supplemental Tables 1-12. No SNP deviated from Hardy-Weinberg expectations. Initially, evidence of genetic association with SCA was identified for forty-five SNPs among 8 genes (SMAD1: 3 SNPs; SMAD3: 12 SNPs; SMAD5: 1 SNP; SMAD6: 6 SNPs; TGFB2: 5 SNPs; TGFBR1: 1 SNP; TGFBR2: 14 SNPs; TGFBR3: 3 SNPs) in the unadjusted analyses (Table 2). Following adjustment for age and sex, nineteen SNPs among 5 genes retained their association with SCA (SMAD1: 2 SNPs; SMAD3: 7 SNPs; SMAD6: 3 SNPs; TGFB2: 2 SNPs; TGFBR2: 5 SNPs). Of note, adjustment for age and sex generally resulted in modest effects on the odds ratio (OR) point estimates. Permutation was employed to account for the number of false positives that are likely given the number of comparisons made, resulting in a single SNP in TGFBR2 (rs9838682) remaining statistically significant with an adjusted $p < 0.05$. An additional SNP in SMAD3 (rs11637581) exhibited a permuted p -value of 0.056 that, although suggestive, did not meet the *a priori* significance threshold for additional analyses (Supplemental Table 3).

TGFBR2 rs9838682 is a G-to-A transition polymorphism with the frequency of the minor allele (A) of 33.1% in controls. The frequency of the “A” allele was significantly elevated in SCA cases (46.6%, $p < 0.001$). The additive genetic model fit the data best. The “A” allele was associated with SCA with an Odds Ratio (OR) of 1.77 (95% Confidence Interval [CI]: 1.28-2.45; $p = 0.0006$). Adjustment for age and sex yielded a similar OR point estimate: 1.66 (95% CI: 1.08-2.54, $p = 0.02$). The association remained statistically significant following permutation testing ($p = 0.045$). Of note, limiting the analysis to the 36 SCA cases occurring during active ischemia (adjusted OR 1.58, 95% CI: 0.89-2.80, $p = 0.12$) or the 53 SCA cases in the absence of ischemia (adjusted OR 1.71, 95% CI: 0.99-2.95, $p = 0.054$) reduced power but resulted in similar point estimates and overlapping confidence intervals for the OR.

TGFBR2 rs9838682 lies in a region of high linkage disequilibrium (LD, Figure 2) in a haplotype block spanning more than 43.6 thousand basepairs (kB). Although other SNPs in this region of LD were associated with SCA (Table 2), none reached statistical significance after permutation testing and were thus not examined in haplotype analyses, as such analyses have limited power to detect meaningful effects.²³ The functional impact of sequence variation in rs9838682 was assessed using PUPASuite 2.0, with no impact predicted for the minor allele.²⁴ Similarly, none of the tested SNPs in LD ($D' \geq 0.80$) with rs9838682 were predicted to impact function.

Discussion

In the present study, we demonstrate an association between a common TGFBR2 polymorphism (rs9838682) and risk of SCA due to malignant ventricular arrhythmias (VA) in the setting of CAD. Rigorously phenotyped cases were collected, with documented presence or absence of CAD and VA. Compared to healthy controls, adjusted for gender and age differences, the rs9838682 minor allele was associated with increased odds of SCA.

The TGFBR2 gene lies on chromosome 3p22 with 7 exons and encodes for the type II TGF β receptor, a 565-amino acid transmembrane tyrosine kinase with a calculated molecular mass of approximately 60 kD.²⁵ In gastrointestinal tissues, TGFBR2 acts as a tumor suppressor gene and approximately 30% of colorectal cancers carry TGFBR2 mutations.²⁶ In the cardiovascular system, mutations resulting in abnormal splicing in TGFBR2 lead to loss of function of TGF β signaling activity on extracellular matrix formation and cause Marfan syndrome.²⁷ Interestingly, tissues derived from affected individuals show increased expression of collagen.

An important substrate for ventricular arrhythmias that leads to SCD post-MI is altered conduction, particularly in the infarct border zone, due to structural remodeling. TGF β plays a key role in the development of cardiac fibrosis.²⁸ In experimental models, ventricular fibrosis increases the incidence of VF initiated by triggered activity.²⁹ After MI, TGF β signaling is altered and plays a central role in infarct healing and cardiac remodeling.¹³ TGF β 1 and TGF β 2 are induced early after MI, while TGF β 3 shows delayed and prolonged upregulation.^{30, 31} SMAD2, 3, and 4 protein expression is upregulated in the scar and border zone area.³²

Recent work from our laboratory demonstrates mitigation of cardiac fibrosis and reduction in vulnerability to atrial and ventricular arrhythmias with an antifibrotic agent (pirfenidone) which is known to inhibit TGF β 1 signaling.³³ TGFBR2 binds all three TGF β ligands, and represents the first critical intracellular step in this complex signaling cascade.¹⁷ Genetic variation in TGFBR2 that may affect function may be expected to play a central role in altered downstream effects, such as enhancing collagen and extracellular matrix synthesis,³⁴ and inducing conversion of fibroblasts to myofibroblasts,³⁵ and thus risk for VT/VF, particularly in the setting of CAD.

The TGFBR2 rs9838682 SNP found to be associated with SCA in the setting of CAD is a common polymorphism. This SNP is intronic, lying between exons 3 and 4, thus it may be in LD with the actual functional variant, or it may impact gene splicing, transcription, or regulation. However, limited data are available on its specific functional effects, therefore further study is necessary to fine-map the association signal, identify causative functional variants, and elucidate their impact on TGFBR2 function or levels, downstream signaling, and whether they ultimately affect development of ventricular arrhythmias.

There are notable limitations to our study. We examined 12 genes in the TGF β signaling pathway but a number of other genes, in particular TGF β 1, in the complex cascade were not explored. In addition, we selected only common SNPs captured on the Affymetrix 6.0 DNA SNP Array, thus rarer polymorphisms and insertion/deletion variants were not assessed by our study. Stringent control for the number of statistical tests substantially reduces power, in particular given the number of SCA cases, such that some associations in the examined SNPs may have been missed. Furthermore, because only Caucasian cases and controls were examined, further studies are needed to determine whether the association with TGFBR2 SNP can be generalized to SCAs in other ethnicities. Healthy controls were used, thus a confounding association with CAD or other intermediate phenotypes such as CHF or hypertension cannot be ruled out. These results may also represent an association between SCA and other variants (in TGFBR2 or other genes) in LD with this SNP. Finally and most importantly, the possibility that this association is due to chance should be considered, thus validation of this finding in independent populations is critical.

The strengths of our study include the rigorously phenotyped and adjudicated SCA cases, compared to healthy controls without SCD. Because of our requirement for documented VA in all SCA cases, the possibility of association with other causes of sudden death is avoided. Because we used shared controls, principal components analysis was employed to exclude the possibility of population stratification as a cause of spurious association.

Conclusion

In summary, our results suggest that genetic variation in TGFBR2 may be associated with risk for SCA associated with CAD. These findings contribute to accumulating evidence for the influence of TGF β signaling in fibrosis and risk for malignant ventricular arrhythmias. If these results are validated, further investigation of the TGF β pathway in the development of ventricular arrhythmias is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary of Abbreviations

SNP, single nucleotide polymorphism
 LD, linkage disequilibrium
 TGF β , Transforming growth factor beta (β)
 VA, ventricular arrhythmia
 VT, ventricular tachycardia
 VF, ventricular fibrillation
 SCA, sudden cardiac arrest
 SCD, sudden cardiac death
 ICD, implantable cardioverter-defibrillator
 CAD, coronary artery disease
 MI, myocardial infarction
 CHF, congestive heart failure
 OR, odds ratio
 CI, confidence interval

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Figure 1.

Gene selection in the TGFβ signaling pathway. Schematic representation of genes examined in the TGFβ signaling pathway. The SMAD mediators can be divided into two main intracellular pathways: SMAD2/3 or SMAD1/5.¹⁷ Members of the TGFβ family (TGFβ1/2/3) bind TGFβR2, Type 2 receptor, a specific serine/threonine receptor kinase which catalyzes the phosphorylation of Type 1 receptor, TGFβR1, activating the protein. TGFβ Type 3 receptor, TGFβR3, may enhance the binding of TGFβ ligands to TGFβR2 by binding TGFβ and presenting it to TGFβR2. Activated type I receptors phosphorylate specific receptor regulated R-SMADs, the intracellular effectors of TGFβ signaling, either SMAD2/3 or SMAD1/5. Activated R-SMADs form heteromeric complexes with SMAD4 that translocate to the nucleus, where they regulate the expression of target genes in conjunction with transcription factors. Inhibitory SMADs, SMAD6 and SMAD7, antagonize TGFβ signaling by inhibiting activation of R-SMADs. We examined twelve genes (TGFB2, TGFB3, TGFBR1, TGFBR2, TGFBR3, SMAD1, SMAD2, SMAD3, SMAD4, SMAD5, SMAD6, SMAD7) in the TGFβ signaling pathway to examine for association with risk of SCA. TGFβ1 was not included since the only variant on the platform did not meet our criteria for a common variant (allele frequency of <0.05) and thus was not analyzed.

Figure 2.

Linkage disequilibrium map of TGFBR2 Gene. D' values are displayed within each diamond. Color scheme gradient indicates r^2 values with red indicating complete LD ($D' = 1.0$) to shades of red to pink ($D' < 1.0$). Blue and white diamonds indicate LD values with suboptimal Log of Odds ($LOD < 2$) scores. Haplotype blocks are defined in black. rs9838682 is an intronic SNP located within the 1st haplotype block of the gene and is indicated by the arrow.

Table 1

Demographic Characteristics of SCA Cases and Healthy Controls

Characteristics	SCA Cases	All Controls	Narcolepsy Controls	Renal Donor Controls	Hap Map Controls
N =	89	520	216	244	60
Age (years)	73.0±12.7	52.7±15.6	59.0±7.5	41.9±13.8	74.0±7.9
Male (%)	90.9	51.9	52.3	52.1	50.0
Congestive Heart Failure (%)	76.5%	N/A			
Ejection Fraction (%)	35.7±14.5	N/A			
Age at index MI (years)	56.7±13.6	N/A			
Hypertension (%)	84.2	N/A			
Diabetes (%)	28.4	N/A			
Current Smoker (%)	10.9	N/A			
Body Mass Index (kg/m ²)	25.7±4.4	N/A			

Table 2

TGFB Pathway SNPs Associated with SCA.

Gene	SNP	Chr	Position	OR (95% CI)	p value	OR _{ADJ} (95% CI)	p value
SMAD1 [*]	rs17797449	4	146588236	0.23 (0.09-0.59)	0.0020	0.31 (0.11-0.85)	0.0237
SMAD1	rs13109195	4	146589206	1.79 (1.03-3.11)	0.0398	2.09 (0.97-4.52)	0.0600
SMAD1 [*]	rs11100883	4	146670420	0.36 (0.17-0.76)	0.0074	0.39 (0.16-0.93)	0.0329
SMAD3	rs12916733	15	65181438	0.60 (0.41-0.88)	0.0099	0.57 (0.35-0.95)	0.0295
SMAD3	rs11637581	15	65199103	0.52 (0.34-0.78)	0.0017	0.52 (0.31-0.87)	0.0123
SMAD3 [*]	rs16950635	15	65205259	1.94 (1.07-3.52)	0.0281	2.17 (1.01-4.63)	0.0463
SMAD3 [*]	rs2033785	15	65228920	3.39 (1.58-7.32)	0.0018	3.86 (1.32-11.3)	0.0135
SMAD3 [†]	rs731874	15	65233885	0.53 (0.33-0.84)	0.0069	0.62 (0.35-1.10)	0.1034
SMAD3 [*]	rs12441344	15	65234949	3.05 (1.38-6.71)	0.0057	2.77 (0.96-8.03)	0.0607
SMAD3	rs17228212	15	65245693	0.50 (0.30-0.84)	0.0079	0.58 (0.31-1.07)	0.0802
SMAD3 [*]	rs11639295	15	65247811	2.47 (1.27-4.79)	0.0074	2.49 (1.05-5.89)	0.0378
SMAD3	rs12913547	15	65254561	1.64 (1.13-2.37)	0.0096	1.68 (1.05-2.71)	0.0320
SMAD3	rs17181556	15	65263822	1.48 (1.04-2.10)	0.0284	1.55 (0.99-2.43)	0.0534
SMAD3	rs12595334	15	65272210	1.60 (1.12-2.28)	0.0094	1.72 (1.09-2.71)	0.0206
SMAD3	rs11556090	15	65273437	1.43 (1.03-1.97)	0.0309	1.26 (0.84-1.91)	0.2658
SMAD5	rs6596283	5	135452251	0.30 (0.11-0.83)	0.0210	0.32 (0.10-1.03)	0.0555
SMAD6	rs16950046	15	64677958	1.79 (1.03-3.11)	0.0386	2.47 (1.17-5.19)	0.0173
SMAD6	rs4293326	15	64923098	0.68 (0.46-1.00)	0.0473	0.75 (0.48-1.18)	0.2119
SMAD6	rs17208967	15	65012648	1.51 (1.09-2.10)	0.0136	1.25 (0.81-1.92)	0.3182
SMAD6	rs17169032	15	65046953	1.60 (1.04-2.45)	0.0319	1.51 (0.85-2.68)	0.1630
SMAD6	rs16950516	15	65099509	0.61 (0.39-0.97)	0.0356	0.49 (0.28-0.84)	0.0106
SMAD6	rs266336	15	65109359	0.60 (0.38-0.96)	0.0323	0.48 (0.28-0.84)	0.0104
TCFB2	rs10482751	1	216622920	1.40 (1.01-1.94)	0.0411	1.77 (1.18-2.67)	0.0062
TCFB2 [*]	rs11118103	1	216646267	4.55 (1.00-20.7)	0.0498	7.15 (1.00-51.3)	0.0504
TCFB2	rs2799083	1	216648240	0.66 (0.45-0.98)	0.0381	0.73 (0.47-1.13)	0.1586
TCFB2	rs10863399	1	216693299	0.57 (0.36-0.92)	0.0210	0.54 (0.31-0.93)	0.0251
TCFB2	rs11118109	1	216699548	0.64 (0.42-0.98)	0.0392	0.61 (0.37-1.00)	0.0522
TCFBR1	rs10819641	9	100976052	1.76 (1.03-3.03)	0.0399	1.75 (0.86-3.56)	0.1246
TCFBR2	rs1495581	3	30322208	1.43 (1.02-2.00)	0.0381	1.44 (0.94-2.20)	0.0951
TCFBR2	rs12486123	3	30355841	0.49 (0.25-0.98)	0.0425	0.57 (0.27-1.22)	0.1496
TCFBR2	rs12489521	3	30359069	1.79 (1.23-2.62)	0.0025	1.71 (1.05-2.78)	0.0299
TCFBR2	rs983682	3	30365871	1.77 (1.28-2.45)	0.0006	1.66 (1.08-2.54)	0.0201
TCFBR2	rs11129399	3	30370955	2.26 (1.28-4.01)	0.0050	1.39 (0.65-2.99)	0.3925
TCFBR2	rs17025355	3	30377499	2.06 (1.14-3.72)	0.0169	1.34 (0.60-2.99)	0.4730
TCFBR2	rs13316253	3	30390272	0.46 (0.22-0.92)	0.0295	0.44 (0.18-1.07)	0.0695
TCFBR2	rs4955316	3	30390616	0.47 (0.23-0.95)	0.0346	0.44 (0.18-1.07)	0.0707
TCFBR2	rs13073411	3	30457314	1.64 (1.11-2.42)	0.0138	1.39 (0.84-2.30)	0.1962
TCFBR2	rs12631561	3	30458371	1.60 (1.08-2.36)	0.0188	1.37 (0.84-2.25)	0.2097
TCFBR2	rs1078985	3	30665915	0.57 (0.38-0.85)	0.0056	0.51 (0.32-0.83)	0.0066
TCFBR2	rs3773634	3	30672940	0.63 (0.43-0.92)	0.0174	0.55 (0.34-0.88)	0.0125
TCFBR2	rs1551762	3	30681394	1.58 (1.07-2.32)	0.0212	1.33 (0.82-2.16)	0.2466
TCFBR2	rs3773644	3	30687348	0.63 (0.45-0.88)	0.0076	0.56 (0.37-0.85)	0.0063
TCFBR3	rs284871	1	91939529	2.03 (1.14-3.62)	0.0155	2.01 (0.91-4.46)	0.0840
TCFBR3	rs10874909	1	91964971	1.91 (1.05-3.46)	0.0336	2.03 (0.91-4.53)	0.0837
TCFBR3	rs2591068	1	92023073	1.89 (1.10-3.24)	0.0208	1.65 (0.83-3.26)	0.1513

Unless otherwise indicated, an additive genetic model fit the data best.

Statistically significant age- and sex-adjusted associations ($p < 0.01$) are listed in bold font. Statistical associations that remained significant following permutation are shaded in grey. Abbreviations: SNP, single nucleotide polymorphism; Chr, chromosome; Position, nucleotide position; OR, odds ratio; CI, confidence interval; OR_{ADJ}, odds ratio adjusted for age and sex.

* A recessive genetic model fit the data best.

† A dominant genetic model fit the data best.