

Polymorphisms in the GNAS Gene as Predictors of Ventricular Tachyarrhythmias and Sudden Cardiac Death: Results From the DISCOVERY Trial and Oregon Sudden Unexpected Death Study

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Background—Population-based studies suggest that genetic factors contribute to sudden cardiac death (SCD).

Methods and Results—In the first part of the present study (Diagnostic Data Influence on Disease Management and Relation of Genetic Polymorphisms to Ventricular Tachyarrhythmia in ICD Patients [DISCOVERY] trial) Cox regression was done to determine if 7 single-nucleotide polymorphisms (SNPs) in 3 genes coding G-protein subunits (GNB3, GNAQ, GNAS) were associated with ventricular tachyarrhythmia (VT) in 1145 patients receiving an implantable cardioverter-defibrillator (ICD). In the second part of the study, SNPs significantly associated with VT were further investigated in 1335 subjects from the Oregon SUDS, a community-based study analyzing causes of SCD. In the DISCOVERY trial, genotypes of 2 SNPs in the GNAS gene were nominally significant in the prospective screening and significantly associated with VT when viewed as recessive traits in post hoc analyses (TT vs CC/CT in c.393C>T: HR 1.42 [CI 1.11–1.80], $P=0.005$; TT vs CC/CT in c.2273C>T: HR 1.57 [CI 1.18–2.09], $P=0.002$). TT genotype in either SNP was associated with a HR of 1.58 (CI 1.26–1.99) ($P=0.0001$). In the Oregon SUDS cohort significant evidence for association with SCD was observed for GNAS c.393C>T under the additive ($P=0.039$, OR=1.21 [CI 1.05–1.45]) and recessive ($P=0.01$, OR=1.52 [CI 1.10–2.13]) genetic models.

Conclusions—GNAS harbors 2 SNPs that were associated with an increased risk for VT in ICD patients, of which 1 was successfully replicated in a community-based population of SCD cases. To the best of our knowledge, this is the first example of a gene variant identified by ICD VT monitoring as a surrogate parameter for SCD and also confirmed in the general population.

Clinical Trial Registration—URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT00478933. (*J Am Heart Assoc.* 2016;5:e003905 doi: 10.1161/JAHA.116.003905)

Key Words: arrhythmia • G proteins • implantable cardioverter-defibrillator • single nucleotide polymorphism • sudden cardiac death • ventricular tachycardia arrhythmia

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Accompanying Tables S1 through S5, Figures S1 through S9 and Appendix S1 are available at <http://jaha.ahajournals.org/content/5/11/e003905/DC1/embed/inline-supplementary-material-1.pdf>

A complete list of the Episode Review Committee, the Adverse Event Advisory Committee and Participating Investigators can be found in the Supplemental Material. Some aspects of the DISCOVERY portion of the study have been presented at the American College of Cardiology 64th Annual Scientific Session & Expo, March 14 to 16, 2015 in San Diego, CA, at the Heart Rhythm Society 36th Annual Scientific Sessions, May 13 to 15, 2015 in Boston, MA, and during a Basic & Translational Science Hot Line Session at the European Society of Cardiology Congress, August 29 to September 2, 2015 in London, England.

The Oregon SUDS findings presented in this manuscript were presented during a Basic & Translational Science Hot Line Session at the European Society of Cardiology Congress, August 29 to September 2, 2015 in London, England.

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Received June 16, 2016; accepted September 21, 2016.

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Ventricular tachyarrhythmias (VT) are one of the leading causes of sudden cardiac death (SCD).¹ Several factors including reduced left ventricular function,² scar burden,³ and myocardial ischemia⁴ promote the occurrence of ventricular tachycardia and ventricular fibrillation. On the other hand, observational studies suggest a distinct contribution of inherited factors to the risk of SCD.^{5,6} This has stimulated the search for genetic variants that may increase susceptibility to SCD.^{7,8} Only recently has the focus been expanded from classical ion channelopathies to other potential mechanisms contributing to arrhythmogenesis. The G-protein signaling pathway is regarded as an appealing candidate in this scenario. G-proteins interact with stimulated adrenoceptors and angiotensin II receptors, thereby initiating diverse intracellular signaling cascades that control cardiovascular pathways.⁹⁻¹¹ Numerous ion channels in myocardial cells interact directly or indirectly with activated G-protein subunits.^{12,13}

Patients who have congestive heart failure with reduced left ventricular function are at increased risk for VT. Implantable cardioverter-defibrillators (ICDs) have been shown to be effective in both primary and secondary prevention of SCD in these patients.^{14,15} We hypothesized that functionally relevant single-nucleotide polymorphisms (SNPs) in genes encoding G-protein subunits may influence the susceptibility to VT, comprising ventricular tachycardia and fibrillation, in patients receiving ICDs for primary prevention indications. To this end, 7 functionally well-defined SNPs in 3 candidate genes—GNB3, GNAQ, and GNAS—coding for G-protein subunits were genotyped in patients with ischemic and nonischemic heart disease receiving ICDs.

Although ventricular arrhythmias recorded by a device do not directly correspond with SCD, they can serve as a surrogate parameter. Thus, any gene variants that were found to predict increased risk of VT in ICD patients would ideally require validation in patients who suffered actual SCD events. Therefore, significantly associated SNPs were further investigated in the Oregon Sudden Unexpected Death Study (Oregon SUDS), a community-based study of SCD.

Methods

The DISCOVERY Study: Study Overview and Patients

The DISCOVERY (Diagnostic Data Influence on Disease Management and Relation of Genetic Polymorphisms to Ventricular Tachyarrhythmia in ICD Patients; ClinicalTrials.gov Identifier NCT00478933) study is a prospective, multicenter longitudinal study designed to investigate the associations of functionally relevant SNPs with the occurrence of ventricular

arrhythmias in patients receiving ICDs for primary prevention. The study was designed by a steering committee. The protocol was approved by the Ethics Committee of each study site, and all patients provided signed informed consent. A total of 1223 patients in 91 European centers were included in the study between April 2007 and June 2011. Patients who were at least 18 years of age were included if they had ischemic or nonischemic heart disease and met approved criteria according to the guidelines for primary prevention with an ICD.¹⁶ The study design has been published previously in detail.¹⁷

DISCOVERY Study: Device Programming and Genetic Analysis

Blood samples (20 mL) were collected from each patient during the initial implant procedure and shipped directly to the genetic core laboratory (Eurofins Medigenomix GmbH, Ebersberg, Germany). Samples were analyzed for 7 SNPs in 3 genes coding for G-protein subunits, including 1 SNP in GNB3: c.825C>T (rs5443); 3 SNPs in GNAQ: c.–909/–908GC>TT (rs72466452), c.–382G>A (rs72466454), c.–387G>A (rs72466453); and 3 SNPs in GNAS: c.393C>T (rs7121), c.2273C>T (rs12481583), c.2291C>T (rs6026584). Instead of the GNAS c.–1211G>A polymorphism, which was described in the method paper,¹⁷ the c.2273C>T polymorphism was genotyped, as both polymorphisms have been shown to be in complete linkage disequilibrium, and c.2273C>T was less prone to genotyping error.¹⁸ Positional and minor allele frequency information for GNAS c.393C>T (rs7121) and GNAS c.2273C>T (rs12481583) were retrieved from the Database of Single Nucleotide Polymorphisms (dbSNP).¹⁹

From April 2007 until April 2009 the protocol required implantation of dual-chamber ICDs. From May 2009 on, the implantation of single-chamber ICDs was allowed to facilitate increased study enrollment; however, the investigators were strongly recommended to implant dual-chamber devices in order to assure reliable discrimination between ventricular and supraventricular tachyarrhythmias. A small number of patients in the study were implanted with CRT-D (Cardiac Resynchronization Therapy-Defibrillator) devices. Programming for detection of arrhythmias was recommended similar to that applied in the EMPIRIC study.²⁰ Criteria for ventricular tachycardias were fulfilled when 16 consecutive beats had a cycle length ≤ 400 milliseconds, and ventricular fibrillation episodes when 18 out of 24 beats had a cycle length ≤ 300 milliseconds.

DISCOVERY Study: Follow-Up and Endpoints

All subjects were followed until at least 1 of the following were met: (1) patients reached their 24 months of follow-up

or (2) the study follow-up closure date, or (3) they exited the study for other reasons. Clinical data were collected at the time of subjects' scheduled follow-up visits at 6, 12, 18, and 24 months after device implantation, at unscheduled follow-ups, system modifications, and subject exit (including deaths) as applicable.

All reported adverse events and deaths were reviewed by the investigators. All reported deaths were reviewed and classified by an Adverse Event Advisory Committee (AEAC), an independent physician committee. Primary endpoints were adjudicated by an independent Episode Review Board (ERB), not by the investigators. The ERB was a blinded committee that classified all reported spontaneous ventricular arrhythmia from device-based EGMs during the clinical study. The blinded review of each reported arrhythmia was performed by at least 2 physicians. In case of mismatched reviews, a third reviewer was involved, and the classification would be a matching of 2 out of 3.

The predefined primary endpoint of the DISCOVERY study was the time to first ventricular arrhythmia, 16 consecutive beats with a cycle length ≤ 400 milliseconds (≥ 150 bpm).

DISCOVERY Study: Statistical Analysis

Data were collected for each patient and recorded on electronic Case Report Forms (eCRF) in a TrialXS database (ClinSource, Brussels, Belgium), programmer printouts, and Save-to-Disk data files.

Baseline characteristics are presented as frequency (percentage) for categorical data and mean (standard deviation) for continuous data unless otherwise stated. Comparison between groups was made using the Fisher exact test for categorical variables, the Cochran-Armitage test for ordinal data, and unpaired t tests for continuous variables.

All subjects successfully genotyped for all 7 study SNPs and with complete device interrogation information from implant to final follow-up time or first VT event were included in the analysis (N=1145; Figure 1). Time to first ventricular tachycardia event was modeled with Cox regression initially using the number of copies of the minor allele for each genotype as ordinal variables (additive model), as prospectively planned. Once evidence of a recessive effect was observed, the recessive model was tested in post hoc analyses. Univariable analyses of VT incidence in relation to baseline characteristics were applied using Cox regression, and missing values were excluded from analysis. In subsequent multivariable analyses, missing values were imputed, as described below. Family-wise error rate was controlled with Bonferroni correction in the 7 prospectively planned tests for the additive effects of the 7 individual SNPs but was not controlled in post hoc analyses. SNPs with adjusted $P \leq 0.1$ were further tested in the multivariate Cox regression

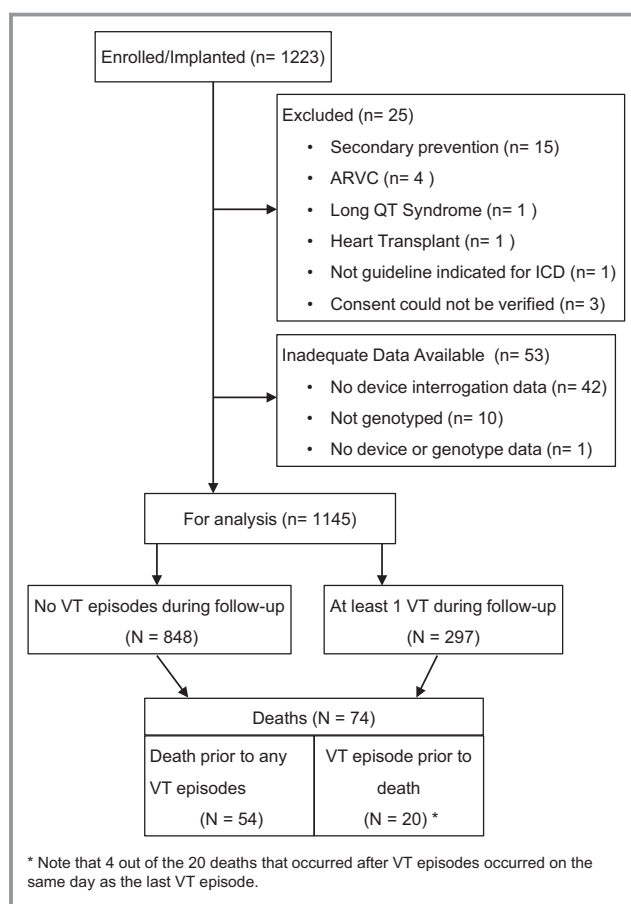


Figure 1. Study overview. ARVC indicates...; ICD, implantable cardioverter-defibrillator; VT, ventricular tachyarrhythmias

model,²¹ along with preselected covariates suspected as potential risk factors for ventricular arrhythmic events—age, left ventricular ejection fraction (LVEF), spontaneous QRS width, and New York Heart Association (NYHA) classification modeled as continuous/ordinal variables and sex, reported history of cardiomyopathy, syncope, atrial fibrillation, non-sustained ventricular tachycardia (NSVT), premature ventricular complexes (PVC), diabetes mellitus and β -blocker or antiarrhythmic medications as dichotomous variables. Note that the reported history variables refer to events that occurred prior to ICD implantation. It is of particular importance that history of NSVT and PVC reflect events that were recorded prior to ICD implantation. Interactions between the SNPs and the nongenetic covariates were explored, but only the interaction between history of cardiomyopathy and GNAS c.393C>T was determined statistically significant and included in the multivariate model. Multiple imputation was performed using the fully conditional specification method to handle missing data with 15 imputations generated, and Rubin's rules were used to combine multivariate regression across imputations.^{21,22} None of the genotype or ventricular event outcome data

were missing for the subjects included in the analysis. Of the covariates that were explored in multivariable modeling, the number missing was 134 (12%) for history of cardiomyopathy, 99 (9%) for QRS width, 25 (2%) for LVEF, 12 (1%) for NYHA class, and <1% for β -blocker use (6), antiarrhythmic medication use (6), history of diabetes mellitus (4), history of syncope (1), and history of NSVT (1). Survival curves were generated by the Kaplan-Meier method and compared by Cox proportional hazard analysis (Cox regression). Event times were measured from the date of ICD implantation. The incremental clinical value of SNP results was assessed by *P*-values within the multivariate Cox Regression modeling and by comparing the hazard ratios for the SNPs to those of the nongenetic covariates in the model. The value of using the combination of the 2 GNAS SNPs was explored by comparing nested models with and without the combination of SNP variables and analyzed with a likelihood ratio test adjusting for multiple imputation.²³ Linkage disequilibrium was calculated using the LD function, and Hardy-Weinberg equilibrium was tested at the $P<0.05$ level by a Pearson Chi-squared test implemented via the HWE.chisq function of the R genetics package (version 1.3.8.1).²⁴ Confidence intervals were calculated at the 95% significance level. Data analysis and statistics for the DISCOVERY part of the study were done by Medtronic statisticians. Analysis was performed using SAS (SAS Institute, Cary, NC; version 9.4) and R (version 3.01).

Oregon SUDS: Study Overview and Patients

The effects of associated SNPs in the DISCOVERY trial were further validated in 986 SCD cases and 349 coronary artery disease controls from the Oregon SUDS. The Oregon SUDS is an established community-based study of SCD ongoing for >13 years in the Portland, Oregon Metropolitan area (population ~1 million).²⁵⁻²⁷ SCD cases were identified from multiple sources, including the emergency medical response system, state medical examiner, and local hospitals. SCD was defined as a sudden pulseless condition of likely cardiac etiology occurring with a rapid witnessed collapse or, if unwitnessed, occurring within 24 hours of last being seen alive in the usual state of health. Subjects with terminal illness, drug overdose, or arrest of noncardiac etiology were excluded. Control subjects with CAD were ascertained from the same geographical region. CAD was defined as 50% stenosis of a major coronary artery, physician report of past myocardial infarction (MI), history of percutaneous coronary intervention or coronary artery bypass grafting, or MI by clinical data with any 2 of the following 3: ischemic symptoms, positive troponins/CKMB or acute ischemic ECG changes, or pathologic Q waves on ECG. The study was approved by the Review Boards of the relevant institutions.

Oregon SUDS: Genotyping and Statistical Analysis

Two significantly associated SNPs in the DISCOVERY trial were further investigated in 1335 subjects from the Oregon SUDS. SCD cases and CAD controls were included in this analysis. SCD cases and controls from the Oregon SUDS were genotyped using the Affymetrix Genome-Wide SNP 6.0 array. Genotypes were imputed using MACH,²⁸ as described earlier.²⁹ From the array, full genotype information was available for c.393C>T, and imputed genotypes were available for c.2273C>T for all cases and 100 controls. For SNP GNAS c.2273C>T, imputed data were available for all cases and 100 controls. We genotyped the remaining controls (n=249) and a proportion of the imputed samples (n=99 cases and 13 controls) using the Taqman assay C_26902679_10. The concordance rate between the imputed data and Taqman genotypes based on replicate samples was 99.1%. The call rate for the genotyped samples was 97.6%. To reduce possible confounding from population stratification, all analyses were restricted to white subjects of European descent aged 18 years and older. SNPs were coded using additive (TT=2, CT=1, CC=0) and recessive (TT=1, CT=0, CC=0) genetic models of inheritance. Logistic regression was performed to test for associations between SNPs GNAS c.393C>T and GNAS c.2273C>T and risk of SCD. All models were adjusted for age and sex. Statistical analysis was performed at Cedars Sinai Heart Institute using the SNP and Variation Suite Package (Golden Helix, Bozeman, Montana).

Results

Patient Characteristics

In the DISCOVERY Study, there were 1223 patients enrolled from 91 centers in 12 countries and implanted with a single- or dual-chamber ICD or CRT-D device. In total, 78 patients were excluded from further analysis (Figure 1), so the analysis cohort includes 1145 patients who met inclusion/exclusion criteria and were successfully genotyped and provided device information. Baseline characteristics of the patients in the analysis cohort are shown for the entire cohort, as well as grouped by patients who did or did not experience a VT episode during follow-up (Table 1). There were significant ($P<0.05$) differences in sex, history of nonsustained ventricular tachycardia prior to implantation, cardiomyopathy etiology, QRS width, and device type between those patients who did and did not experience VT episodes during the study follow-up period.

There was a median follow-up time of 575 days (interquartile range 364-730), with collection of device recordings of potential VT events. During the follow-up period, 297 (25.9%) patients experienced at least 1 VT episode. There were 74 deaths during the course of the study.

Table 1. Baseline Characteristics by Ventricular Tachycardia Episode Groups

Variable	VT Episodes			Total Patients (n=1145)
	No (n=848)	Yes (n=297)	Cox Regression P Value	
Age, y	61.7±11.1	61.4±10.3	0.73	61.6±10.9
Body mass index	27.8±4.9	28±4.6	0.70	27.9±4.8
Sex			0.04	
Female	17.3%	11.8%		15.9%
Male	82.7%	88.2%		84.1%
Diabetes mellitus	31.8%	35.4%	0.28	32.7%
Etiology of heart failure			0.004	
Nonischemic	34.2%	44.8%		36.9%
Ischemic	65.8%	55.2%		63.1%
QRS width, ms	106.9±24.5	110.6±23.2	0.04	107.9±24.2
LVEF, %	29.4±10	28.8±10.4	0.21	29.3±10.1
NYHA class			0.23	
I	12.3%	9.8%		11.7%
II	58.7%	60.3%		59.1%
III	27.4%	29.2%		27.9%
IV	1.6%	0.7%		1.3%
Medical history of				
NSVT	24.4%	38.4%	<0.0001	28.1%
Atrial fibrillation	19.8%	19.9%	0.69	19.8%
Hypertension	57.1%	58.2%	0.44	57.4%
PVC	15.5%	19.5%	0.09	16.5%
Syncope	7.9%	9.8%	0.35	8.4%
Medication at enrollment				
ACE inhibitor	84.2%	87.2%	0.27	85.0%
β-Blocker	89.8%	87.8%	0.59	89.3%
Antiarrhythmic	14.7%	13.2%	0.81	14.3%
Diuretic	79.0%	80.7%	0.19	79.5%
Digoxin	10.2%	12.8%	0.13	10.9%
Lipid-lowering drug	69.9%	68.9%	0.43	69.6%
Initial device implant			0.04	
Dual-chamber ICD	64.6%	62.0%		63.9%
Single-chamber ICD	35.4%	38.0%		36.1%

ACE indicates angiotensin-converting enzyme; ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; NSVT, nonsustained ventricular tachycardia; NYHA, New York Heart Association; PVC, premature ventricular complexes.

Single Nucleotide Polymorphisms

Of the 7 SNPs prospectively selected for analysis in the DISCOVERY study, none of the genotype distributions had detectable deviations from Hardy-Weinberg equilibrium. Of the 7 SNPs, GNAS c.393 C>T (HR 1.24; unadjusted $P=0.01$; adjusted $P=0.06$) and GNAS c.2273 C>T (HR 1.28; unadjusted $P=0.003$; adjusted $P=0.02$) were found to have a nominally

significant association with VT incidence, and GNAS c.2273 C>T remained significant after correction for multiple comparison testing (Table S1). Within the analysis population, the 2 significant GNAS SNPs were moderately linked (correlation= -0.277 ; $D'=0.369$). The 2 SNPs are located 9752 nucleotides from each other, and their minor allele frequencies were consistent with reports for white populations (Table S2).¹⁹

When analyzed as nominal variables, both for c.393 C>T and c.2273 C>T, the TT genotype was found to differ from the CC and CT genotypes (Table 2), and the Kaplan-Meier survival plots further illustrate that both SNPs appear to have a recessive TT effect (Figure 2A and B). The Kaplan-Meier estimate for VT incidence at 24 months in 305 patients (26.6%) with TT genotype in c.393 C>T was 41.1% (CI 34.7% to 48.1%), whereas the incidence in patients with CC/CT genotypes was 30.9% (CI 27.3% to 34.9%). The Kaplan-Meier estimate for VT incidence at 24 months in 156 patients (13.6%) with TT genotype in c.2273 C>T was 45.3% (CI 36.7% to 54.8%), and that for patients with CC/CT genotypes was 31.6% (CI 28.2% to 35.3%). This increased risk remained unmodified after inclusion of nongenetic covariates (Table 2) when each SNP was modeled as a recessive effect. When the interaction term between c.393 C>T and ischemic cardiomyopathy etiology was introduced into the model, it was found to be statistically significant ($P=0.0052$). In ischemic patients, there was significantly increased risk of VT with the c.393 C>T TT genotype (HR 1.76 [CI 1.28-2.44], $P=0.0006$) but not in the nonischemic patients (HR 0.84 [CI 0.56-1.26], $P=0.4$). No other interaction terms were found to be significant between either of the SNPs and any of the other covariates included in the multivariate model. Homozygosity for the T allele in at least 1 of c.393 C>T or c.2273 C>T (T_H) was associated with a HR of 1.58 (CI 1.26-1.99) ($P=0.0001$, Figure 2C). The

incremental predictive value of T_H was assessed by comparing models with all non-SNP covariates with and without T_H and found to be statistically significant ($P=0.00055$). Patients having the TT genotype at both c.393 C>T and c.2273 C>T did not have increased risk compared to patients having the TT genotype at only 1 of those 2 loci.

Because VT episodes were treated by implanted devices, it was not possible to fully assess whether the episodes would have been lethal without treatment. As an alternative to determining episode lethality, episodes were classified by whether they were slow (<250 bpm) or fast (≥ 250 bpm) using the corresponding median VT episode cycle length cutoff of 240 milliseconds. Time to first event analysis was performed for each zone (Table 3). There were 280 and 52 patients with at least 1 episode in the slow and fast zones, respectively. T_H was associated with episode incidence in both the slow zone ($P=0.002$; HR=1.46 [CI 1.15-1.85]) and the fast zone ($P=0.008$; HR=2.08 [CI 1.21-3.58]).

Results: Validation Population

The mean ages of SCD and CAD controls from the Oregon SUDS were 60.5 ± 12.9 and 65.2 ± 10.9 years, respectively; 72% of cases and 68% of controls were male.

Significant evidence for association was observed for SNP GNAS c.393C>T under the additive ($P=0.039$, OR=1.21 [1.05-

Table 2. c.393C>T and c.2273C>T Association With Time to First VT Event

Parameter	Comp Group	Univariate		Multivariate	
		P Value	HR	P Value	HR
NSVT	Yes	<0.0001	1.80 (1.42–2.27)	<0.0001	1.89 (1.46–2.46)
Ischemic cardiomyopathy	Yes	0.004	0.70 (0.55–0.89)	0.01	0.70 (0.54–0.91)
QRS width		0.04	1.00 (1.00–1.01)	0.03	1.01 (1.00–1.01)
c.2273C>T (TT vs CC/CT)	TT	0.002	1.57 (1.18–2.09)	0.03	1.40 (1.04–1.89)
Sex	Male	0.04	1.45 (1.02–2.06)	0.04	1.47 (1.02–2.10)
LVEF		0.21	0.99 (0.98–1.00)	0.05	0.99 (0.97–1.00)
c.393C>T (TT vs CC/CT)	TT	0.005	1.42 (1.11–1.80)	0.05	1.29 (1.00–1.66)
Anti-arrhythmics	Yes	0.81	0.96 (0.69–1.34)	0.08	0.73 (0.51–1.04)
NYHA class		0.22	1.11 (0.94–1.31)	0.23	1.11 (0.93–1.33)
Diabetes mellitus	Yes	0.28	1.14 (0.90–1.45)	0.25	1.15 (0.90–1.48)
Age		0.73	1.00 (0.99–1.01)	0.47	1.00 (0.98–1.01)
Beta blockers	Yes	0.59	0.91 (0.64–1.29)	0.47	0.88 (0.61–1.25)
Syncope	Yes	0.35	1.20 (0.82–1.76)	0.60	1.11 (0.74–1.67)
Atrial fibrillation	Yes	0.69	1.06 (0.80–1.41)	0.87	1.03 (0.76–1.38)
PVC	Yes	0.09	1.28 (0.96–1.71)	0.69	1.06 (0.79–1.43)

Comp group, comparison group (hazard ratio indicates risk of being in the comparison group vs the reference group); Univariate, univariate Cox regression of variable to time to first VT event; Multivariate, individual contribution of variable in a Cox regression with full list of variables in table as covariates in a multivariate model; LVEF indicates left ventricular ejection fraction; NSVT, nonsustained ventricular tachycardia; NYHA, New York Heart Association; PVC, premature ventricular complexes.

1.45]) and recessive ($P=0.01$, $OR=1.52$ [1.10–2.13]) genetic models. We did not find a statistically significant association of SNP c.2273C>T and SCD (Table 4).

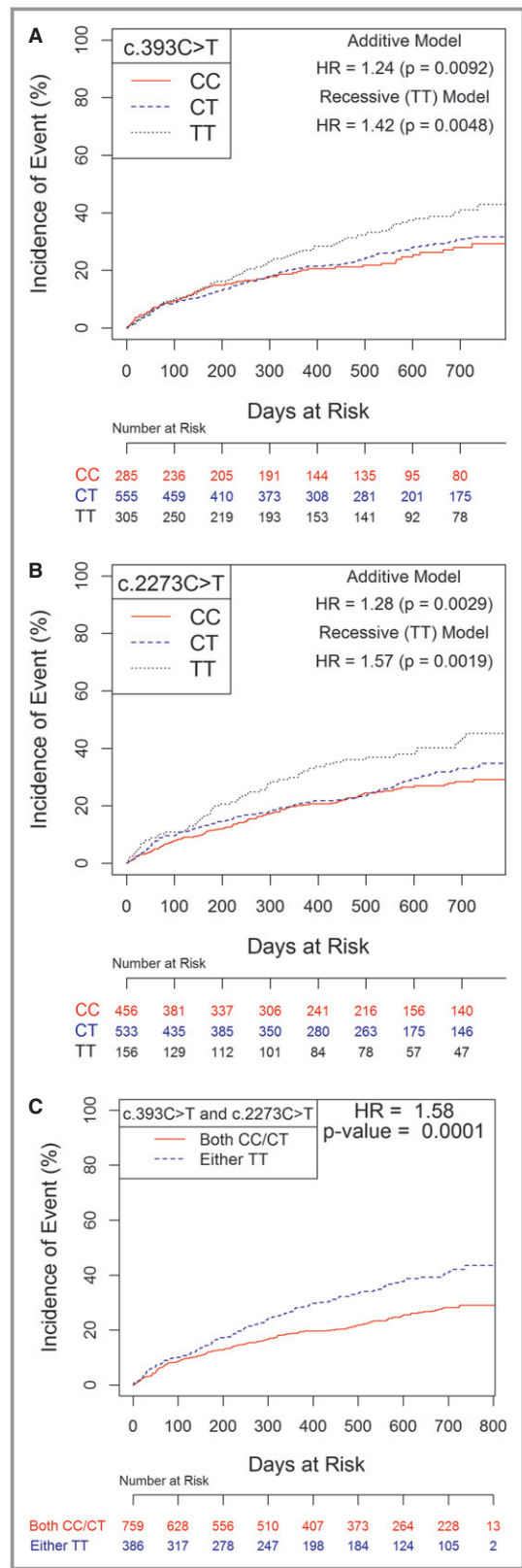


Table 3. Differential Association of SNPs c.393C>T and c.2273C>T With Respect to VT Cycle Length and Shock Delivery

	c.393C>T (TT vs CC/CT)	c.2273C>T (TT vs CC/CT)
Slow VT (400–240 ms) 280 patients with events	1.30 (1.01–1.67) $P=0.043$	1.58 (1.18–2.12) $P=0.002$
Fast VT (≤ 240 ms) 52 patients with events	1.89 (1.08–3.28) $P=0.025$	1.07 (0.50–2.27) $P=0.861$
Shock delivery 100 patients with events	1.66 (1.11–2.50) $P=0.014$	1.41 (0.86–2.33) $P=0.177$

VT indicates ventricular tachyarrhythmia.

Discussion

The DISCOVERY part of this study showed that the gene GNAS harbors 2 SNPs (c.2273C>T and c.393C>T) that were associated with an increased risk for VT identified by ICD, after adjustment for nongenetic risk predictors. Homozygosity for the T allele in either or both SNPs was associated with a hazard ratio of 1.58 for VT. In the validation phase, c.393C>T was successfully replicated to be associated with an increased risk for SCD in a community-based population. To the best of our knowledge this may be the first example of a common genetic variant that was found to be associated with ICD-detected ventricular arrhythmia as a surrogate parameter for SCD and confirmed in the general population. This significant association suggests an important role of $G_{\alpha s}$ -mediated signal transduction in ventricular arrhythmogenesis.

Potential Mechanisms of GNAS Polymorphisms

Sympathetic stimulation of the heart is one of the predominant triggers of fatal or near-fatal ventricular arrhythmias.³⁰ Thereby, sympathetic activation is strongly controlled by β -adrenoceptors that belong to the G-protein-coupled

Figure 2. Kaplan-Meier estimates of time to first VT episodes for c.393C>T (A) and c.2273C>T (B) separately. Hazard ratio (HR) and P -value are shown as derived from a Cox regression model including genotype as ordinal variable, counting the number of T alleles. (C), Kaplan-Meier estimates of time to first VT episodes for combined effects of c.393C>T/c.2273C>T. Hazard ratio (HR) and P -value are shown as derived from a Cox regression model including genotype as ordinal variable, counting the number of T alleles.

Table 4. Association Results for SNPs c.393C>T and c.2273C>T in the Oregon SUDS

SNP*	Minor Allele	Minor Allele Frequency		Additive [†]	Recessive [‡]
		Cases	Controls		
c.393C>T	T	0.47	0.43	1.21 (1.05–1.45) P=0.04	1.52 (1.10–2.13) P=0.01
c.2273C>T	T	0.35	0.37	0.90 (0.81–1.10) P=0.37	0.90 (0.70–1.51) P=0.90

SNP indicates single nucleotide polymorphism; SUDS, Sudden Unexpected Death Study.

*There were 986 cases for both SNPs. There were 349 controls for c.393C>T and 338 controls for c.2273C>T.

[†]Additive mode of inheritance (TT=2, CT=1, CC=0).

[‡]Recessive mode of inheritance (TT=1, CT=0, CC=0).

receptor family. Stimulation of β_1 - and β_2 -adrenoceptors leads to an activation of the stimulatory G-protein that initiates an intracellular signaling.³¹ Excessive adrenoceptor stimulation has been shown to cause severe cardiac arrhythmias.³²

The stimulatory G-protein α -subunit (G_{α_s}) is encoded by the GNAS gene. Sequencing the promoter and intron 1 of GNAS revealed 11 SNPs (including c.2273C>T and c.–1211G>A), resulting in 3 common haplotypes (*1, *2, *3). The A allele of c.–1211G>A is part of a functional GNAS haplotype *3 and is in complete linkage disequilibrium with the T allele of c.2273C>T.¹⁸ The A allele of c.–1211G>A has been shown to enhance transcription factor binding and G_{α_s} protein expression.³³ In addition, it is characterized by enhanced G_{α_s} expression and an increased responsiveness of human heart tissue to β -adrenergic stimulation, which is associated with increased cardiac stroke volume, reduced BNP serum levels, and increased survival after coronary artery bypass surgery.³⁴ Patients carrying GNAS haplotype *3 seem to benefit in particular from β -blocker therapy.³⁵

An increased stimulatory effect has also been observed for the c.393C>T. This polymorphism in exon 5 of the GNAS gene has been shown to be linked to essential hypertension and to responsiveness to β -blocking agents.³⁶ The TT genotype is associated with increased G_{α_s} mRNA expression in heart cell specimens.³⁷ Although the precise role of this SNP in the alteration of intracellular signal transduction is not yet fully understood, the TT genotype seems also to be associated with increased G_{α_s} activity.

Although an increased G_{α_s} expression in relation to the c.393C>T TT genotype or homozygous GNAS haplotype *3 status may be advantageous in terms of increased cardiac contractility, the downside of this effect is that it may be associated with increased catecholaminergic excitability and concomitantly increase lifetime risk for cardiomyopathy and

cardiac arrhythmias. This is illustrated by findings showing that cardiac overexpression of G_{α_s} in mice induces the typical features of cardiomyopathy, including a marked increase in arrhythmias and mortality in older animals.³⁸ Therefore, our findings of an increased risk for VT in carriers of GNAS alleles associated with an increased G_{α_s} expression strongly resemble these findings in transgenic mice.

GNAS is a complex genetic locus with several different gene products and is affected by both imprinting and alternative splicing. Disruption of the expression of its gene products is known to result in a number of different human disorders, including endocrine disorders, and poor prognosis in a variety of different cancer types.³⁹ Given the complexity of the locus, it is possible that there are multiple phenotypical consequences affecting cardiovascular health.

Leveraging Both ICD and Community Cohorts for Investigation of Gene Variants

The use of a population-based cohort for confirmation strengthens the validity of our findings in ICD patients. The patients who meet criteria for primary prevention are likely to be a selected high-risk subgroup identified by a LV ejection fraction (EF) below 35%. However, there is increasing recognition of the fact that the vast majority of SCD cases may not have this critical decrease in LV ejection fraction. Because cases of sudden cardiac arrest ascertained in the Oregon SUDS are an unselected population, patients with LV ejection fraction values that traverse the whole spectrum from severely decreased to preserved ejection fraction were included. Therefore, our findings for c.393C>T are likely to be specific to arrhythmia causing SCD that is dependent on low ejection fraction.

In the DISCOVERY population, the use of the programming methodology of the EMPIRIC trial was recommended to the participating centers.²⁰ With this programming, 297 (25.9%) patients experienced at least 1 VT episode within a median follow-up of 575 days. This programming was done to ensure a high sensitivity for ventricular arrhythmias. However, this also means that our endpoint may not directly indicate true SCD risk. In order to get a more valid surrogate for arrhythmic death, episodes were classified into arrhythmia zones by median cycle lengths. This categorization showed that homozygosity for the T allele in the SNP c.393C>T but not c.2273C>T was a significant predictor of the occurrence of VTs in the fast zone. In addition, only c.393C>T was predictive for shock delivery of the ICD device. These results suggest that c.393C>T is particularly linked to life-threatening arrhythmias. Furthermore, this diverging impact of c.393C>T and c.2273C>T on the occurrence of fast VTs and shock delivery might explain the fact that only c.393C>T but not c.2273C>T was linked to sudden cardiac death in the Oregon SUDS study.

GNAS Polymorphism for Risk Stratification of Ventricular Arrhythmias

Risk stratification of patients for primary prevention of SCD is predominantly based on LV EF. In order to further stratify patients with decreased LV function on the basis of the data of MADIT II, Goldenberg et al developed a risk score model comprising 5 clinical factors (NYHA functional class, atrial fibrillation, QRS-duration, age, and blood urea nitrogen >26 and <50 mg/dL). In a multivariate proportional hazards regression model, HRs between 1.87 (NYHA functional class) and 1.56 (blood urea nitrogen) were calculated.⁴⁰ In the present study homozygosity in the T allele of either c.393C>T or c.2273C>T or both was associated with a HR of 1.58 for ventricular arrhythmias. The present data show that the investigated polymorphisms have comparable predictive value for ventricular arrhythmias as established factors in preselected patients with reduced left ventricular function.

However, the predictive value of all of these metrics, including the polymorphisms under investigation, will need more extensive validation in multiple cohorts prior to being implemented in future risk-stratifying efforts for ICD therapy. Although combining genetic and conventional risk factors might be helpful to improve current risk stratification, a prospective validation should be performed to provide more substantial evidence of their real value in clinical practice.

Strengths and Limitations

There are a number of factors in the study execution that affected the achieved power of the study (Figures S1 through S9). First, the VT incidence rate is lower than expected when the sample size calculation was made. Bernoulli variance is maximal for $P=0.5$ (ie, when half of the subjects experience VT events) and decreases as P increases to 1 or decreases to 0, so our power should be greater than initially expected because both the expected and observed VT incidence rates were <0.5. Alternatively, from April 2007 until April 2009 the protocol required implantation of dual-chamber ICDs. From May 2009 on, the implantation of single-chamber ICDs was allowed to facilitate increased study enrollment. ICDs have a high sensitivity for detecting VT; however, specificity is compromised by difficulties in discriminating between ventricular and supraventricular arrhythmias, especially in single-chamber devices. With the use of dual-chamber devices misclassification of arrhythmias can be considerably reduced.⁴¹ A post hoc analysis of our data revealed that results for the 2 GNAS SNPs were more significant in patients with dual-chamber devices, and GNAQ c.-382G>A emerges as having a statistically significant association with VT, suggesting that use of single-chamber devices led to diminished study power (Tables S3 through S5). It is reasonable to believe that the single-chamber device subset led to higher

misclassification of arrhythmias and, as a result, led to lower power than had the entire cohort been dual-chamber patients. As a result, we remain confident that the 2 GNAS markers lead to differential risk for VT events and are less confident that there are not differences in VT risk in the remaining 5 SNPs. This is particularly true for GNAQ c.-382G>A and GNAQ c.-387G>A, where the minor allele frequency is below 10% in the study cohort.

A major strength of the DISCOVERY study is that it was a prospective analysis of only 7 SNPs, based on their mechanistic role in G-protein-coupled receptor signal transduction. Although many contemporary SNP studies are genome-wide screens, which even with very large sample sizes can miss subtle SNP effects due to the family-wise error correction due to immense number of comparisons, this study benefited from a very small number of targeted SNPs.

DISCOVERY was limited to white European subjects, and the Oregon SUDS population was limited by the population within the Portland metropolitan area. Additional study would be necessary to understand the effects of the GNAS SNPs in more diverse populations.

Conclusion

The present results suggest an important role of G_{α_s} -mediated signal transduction in ventricular arrhythmogenesis. GNAS harbors 2 SNPs that were associated with an increased risk for VT in ICD patients of which 1 was successfully replicated to be associated with an increased risk in a community-based population of SCD cases.

To our knowledge, these results represent the first example of a gene variant that was identified by ICD monitoring in a specific subpopulation and then confirmed in the general population. Thus, these findings have broader clinical implications beyond the currently indicated ICD population and may lead to further understanding of SCD risk mechanisms in the general population.

Sources of Funding

The DISCOVERY Study was funded by Medtronic. The Oregon SUDS study was funded in part by National Heart, Lung, and Blood Institute grants R01HL105170 and R01HL122492 to Dr Chugh.

Disclosures

Dr Wieneke has received Consultant Fees and Honoraria from Biotronik and Medtronic. Dr Svendsen has received consultant fees and honoraria from Medtronic, Biotronik, Boehringer-Ingelheim, and Astra-Zeneca and has received research

grants from Medtronic, Gilead, and Biotronik. Dr Lande is a full-time employee of Medtronic. Dr Spencker has received consultant fees and honoraria from Medtronic. Dr Foldesi has received consultant fees and honoraria from Medtronic, St Jude Medical Inc, Biotronik, and Boston Scientific. Dr Siffert has received consultant fees and honoraria from Eurofins Medigenomix. The other authors have nothing to disclose.

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Supplemental Material

DISCOVERY SNP Details

Table S1. G-Protein Subunit SNP Prediction of Time to First VT/VF Event

Univariate analyses of the 7 studied G-protein subunit SNPs, modelled as additive minor allele effects.

SNP Detail	dbSNP ID	HR	p-values	
			Raw	Bonf
GNAS c.393 C > T	rs7121	1.24	0.01	0.06
GNAS c.2273 C > T	rs12481583	1.28	0.003	0.02
GNAS c.2291 C > T	rs6026584	1.07	0.47	1.00
GNAQ c.-382 G > A	rs72466454	0.78	0.11	0.80
GNAQ c.-387 G > A	rs72466453	1.14	0.50	1.00
GNAQ c.-909/-908 GC > TT	rs72466452	1.05	0.52	1.00
GNB3 c.825 C > T	rs5443	1.01	0.88	1.00

HR = Hazard ratio

Raw = Raw p-values from Cox regression

Bonf = Bonferroni-adjusted p-value

Table S2. Allele Frequencies Major and minor allele dbSNP (build ID 142) frequencies within the DISCOVERY study cohort of the 7 studied G-protein subunit SNPs.

SNP	Allele Frequency		Genotype Frequency *		
	Major	Minor	M/M	M/m	m/m
GNAS c.2273 C > T	63.10%	36.90%	39.83%	46.55%	13.62%
GNAS c.2291 C > T	68.21%	31.79%	46.03%	44.37%	9.61%
GNAS c.393 C > T	49.13%	50.87%	24.89%	48.47%	26.64%
GNB3 c.825 C > T	68.60%	31.40%	46.81%	43.58%	9.61%
GNAQ c.-382 G > A	90.79%	9.21%	82.53%	16.51%	0.96%
GNAQ c.-387 G > A	95.33%	4.67%	90.92%	8.82%	0.26%
GNAQ c.-909/-908 GC > TT	53.71%	46.29%	29.87%	47.69%	22.45%

* M = major allele and m = minor allele

Single Chamber and Dual Chamber Subgroup Analyses

Subjects in the DISCOVERY cohort were separated into those with single and those with dual chamber devices. Although some subjects had device change-outs during their follow-up periods, there were none who changed from single to dual or dual to single during study follow-up. There were 413 subjects with single chamber devices and 732 subjects with dual chamber devices. Time to first VT event was analyzed by Cox Regression for all 7 SNPs in both subgroups and the results are shown in the tables below.

Table S3. Single Chamber Device Subjects (n=413) Univariate analyses of the 7 studied G-protein subunit SNPs for subjects implanted with single chamber devices.

SNP	Additive Model		Dominant (minor allele)		Recessive (minor allele)	
	p-value	HR	p-value	HR	p-value	HR
GNAQ c.-909/-908GC>TT	0.65	1.06	0.93	0.98	0.52	1.15
GNAQ c.-382G>A	0.74	1.08	0.59	1.15	0.64	1.60
GNAQ c.-387G>A	0.83	0.94	0.97	0.99	0.98	164497
GNAS c.2273C>T	0.17	1.20	0.36	1.20	0.16	1.39
GNAS c.393C>T	0.76	1.04	0.59	1.14	0.99	1.00
GNAS c.2291C>T	0.57	1.09	0.83	0.96	0.38	1.36
GNB3 c.825C>T	0.61	0.93	0.95	0.99	0.31	1.49

Table S4. Dual Chamber Device Subjects (n=732) Univariate analyses of the 7 studied G-protein subunit SNPs for subjects implanted with dual chamber devices.

SNP	Additive Model		Dominant (minor allele)		Recessive (minor allele)	
	p-value	HR	p-value	HR	p-value	HR
GNAQ c.-909/-908GC>TT	0.71	1.04	0.71	0.94	0.80	1.04
GNAQ c.-382G>A	0.03	0.64	0.03	0.62	0.88	1.17
GNAQ c.-387G>A	0.32	1.28	0.32	1.28	NA	NA
GNAS c.2273C>T	0.01	1.32	0.08	1.31	0.01	1.67
GNAS c.393C>T	0.003	1.36	0.20	1.26	0.0003	1.73
GNAS c.2291C>T	0.59	1.06	0.26	0.85	0.46	0.84
GNB3 c.825C>T	0.54	1.07	0.49	1.11	0.87	0.96

Table S5. Dual Chamber SNP Details Contingency tables of SNPs significant in Table S4, showing distribution of genotype grouped by whether DISCOVERY subjects experienced a VT event during study follow-up.

GNAS c.393C>T	PE (Primary Endpoint - fast VT)		
	No Event	Event	Total
CC/CT	416 (78%)	116 (22%)	532
TT	132 (66%)	68 (34%)	200
Total	548	184	732
GNAS_2273_TRec			
CC/CT	484 (77%)	147 (23%)	631
TT	64 (63%)	37 (37%)	101
Total	548	184	732
GNAQ_382_ADom			
GG	437 (73%)	159 (27%)	596
G/AA	111 (82%)	25 (18%)	136
Total	548	184	732

Power Estimation Plots

A series of figures are provided below showing the achieved power, given the results in the study and plotted out for a range of values around the value within the study for each of the 7 SNPs in the DISCOVERY study and the two relevant GNAS SNPs in the Oregon-SUDS study. For all estimates, the power is calculated assuming a logistic regression model and a point estimate of the odds ratio (estimated by the reported hazard ratio for the DISCOVERY calculations).

Figure S1. Achieved power of the GNAS c.393 C>T SNP in the DISCOVERY study (additive model) over a range of test odds ratios (OR).

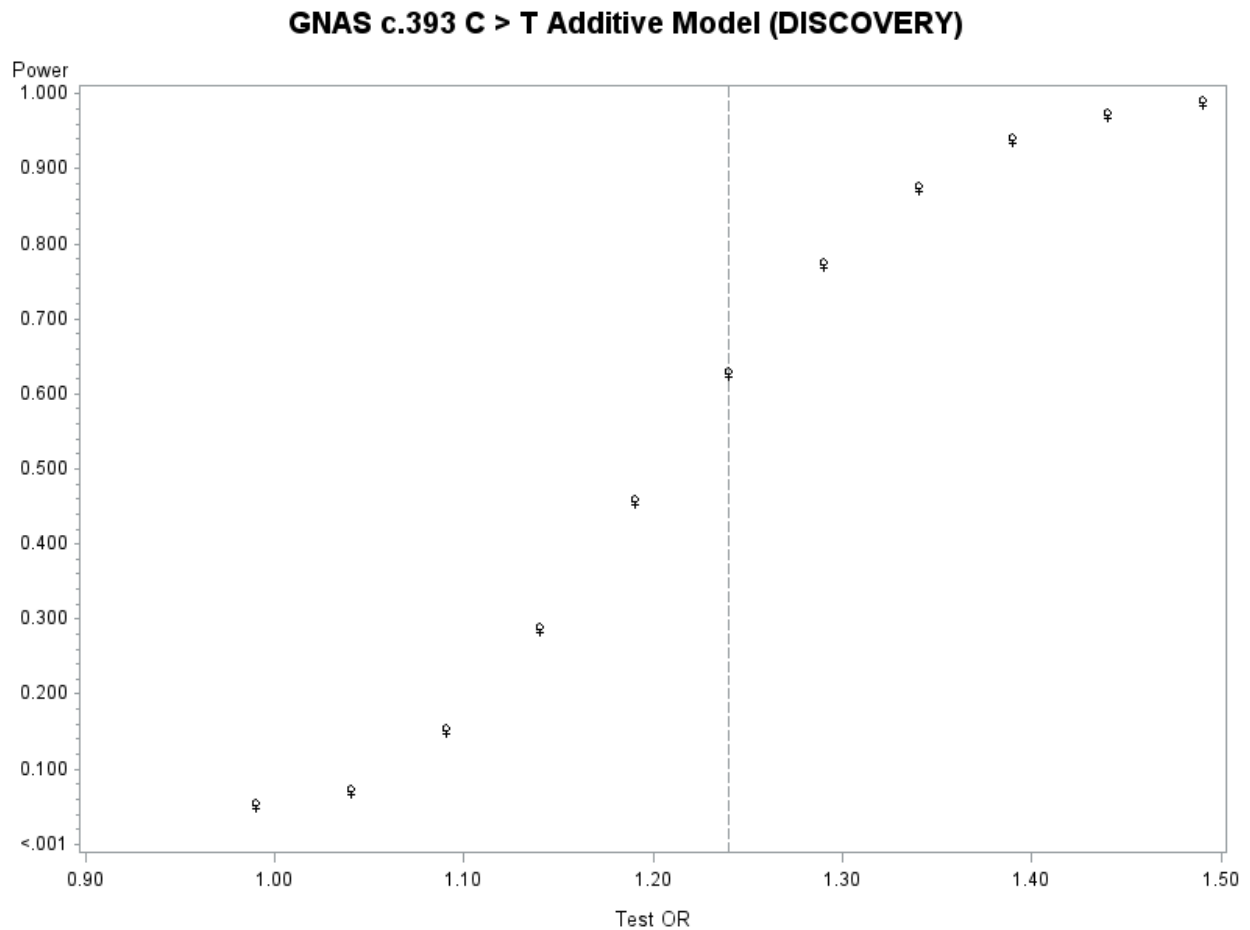


Figure S2. Achieved power of the GNAS c.2273 C>T SNP in the DISCOVERY study (additive model) over a range of test odds ratios (OR).

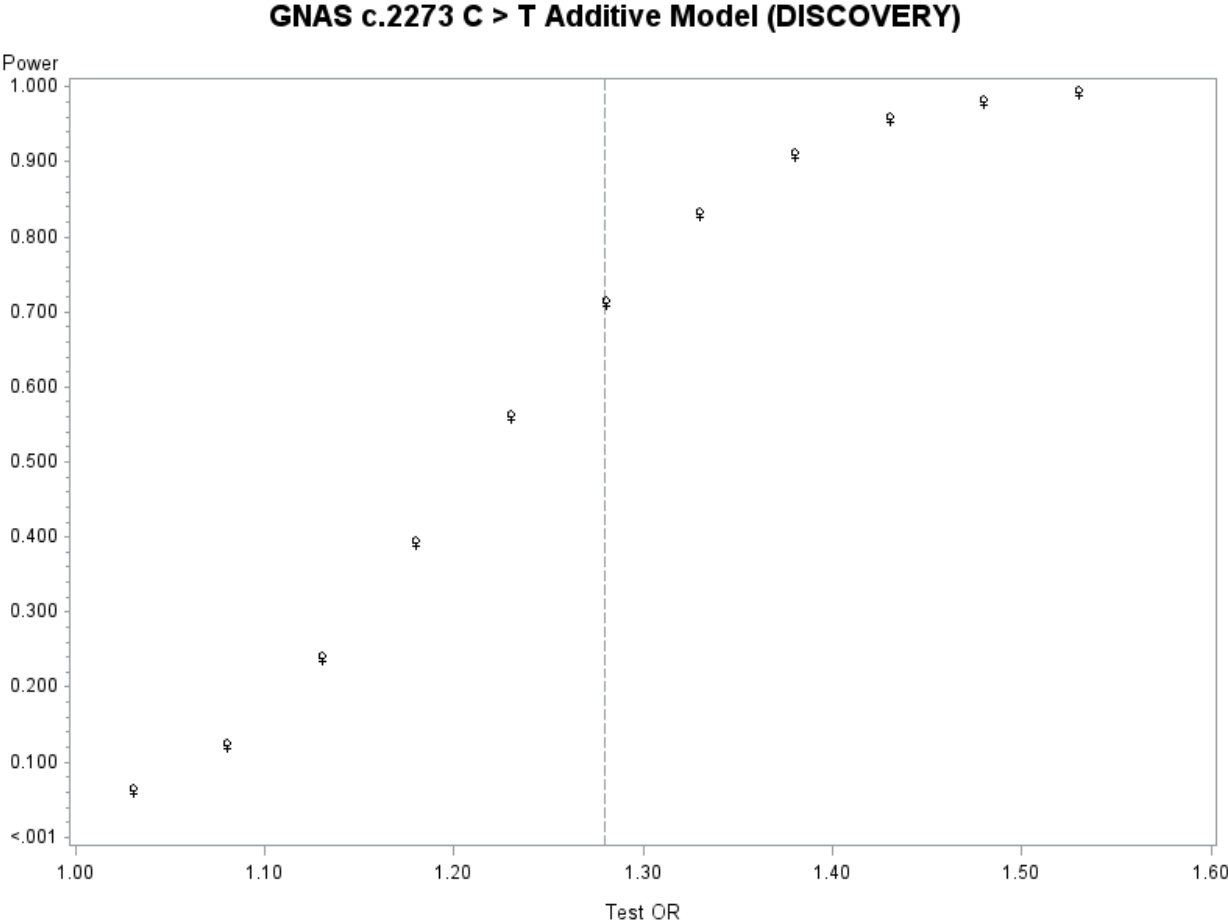


Figure S3. Achieved power of the GNAS c.2291 C>T SNP in the DISCOVERY study (additive model) over a range of test odds ratios (OR).

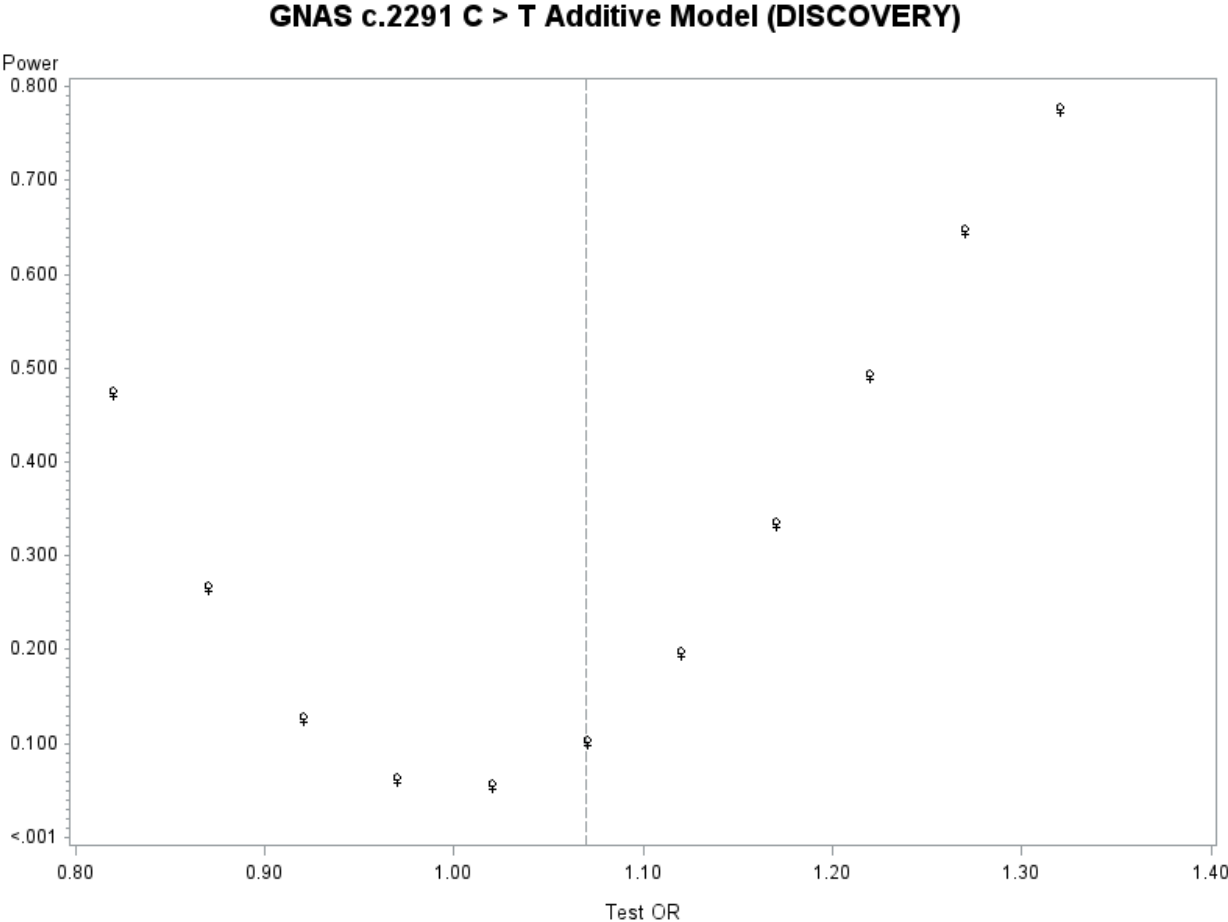


Figure S4. Achieved power of the GNB3 c.825 C>T SNP in the DISCOVERY study (additive model) over a range of test odds ratios (OR).

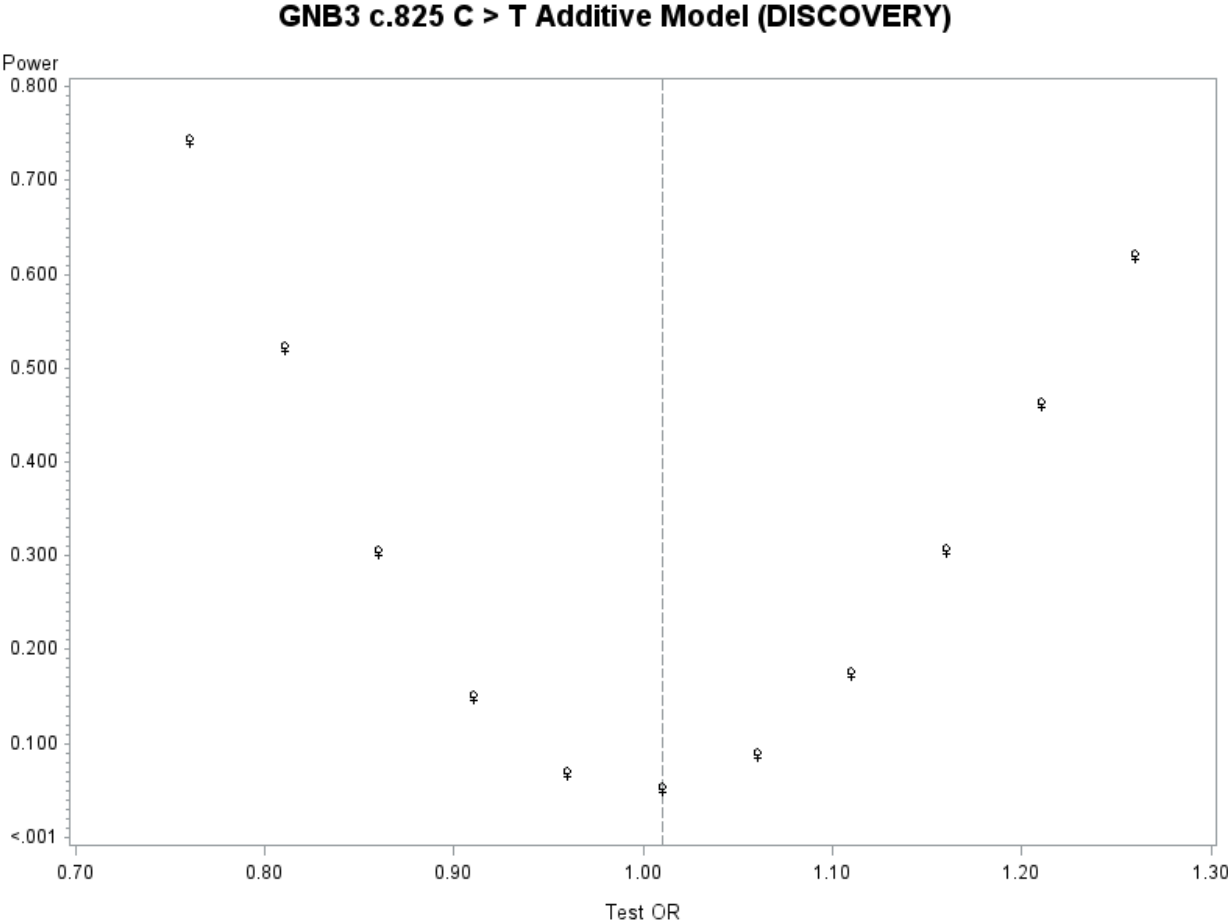


Figure S5. Achieved power of the GNAQ c.-382 G>A SNP in the DISCOVERY study (additive model) over a range of test odds ratios (OR).

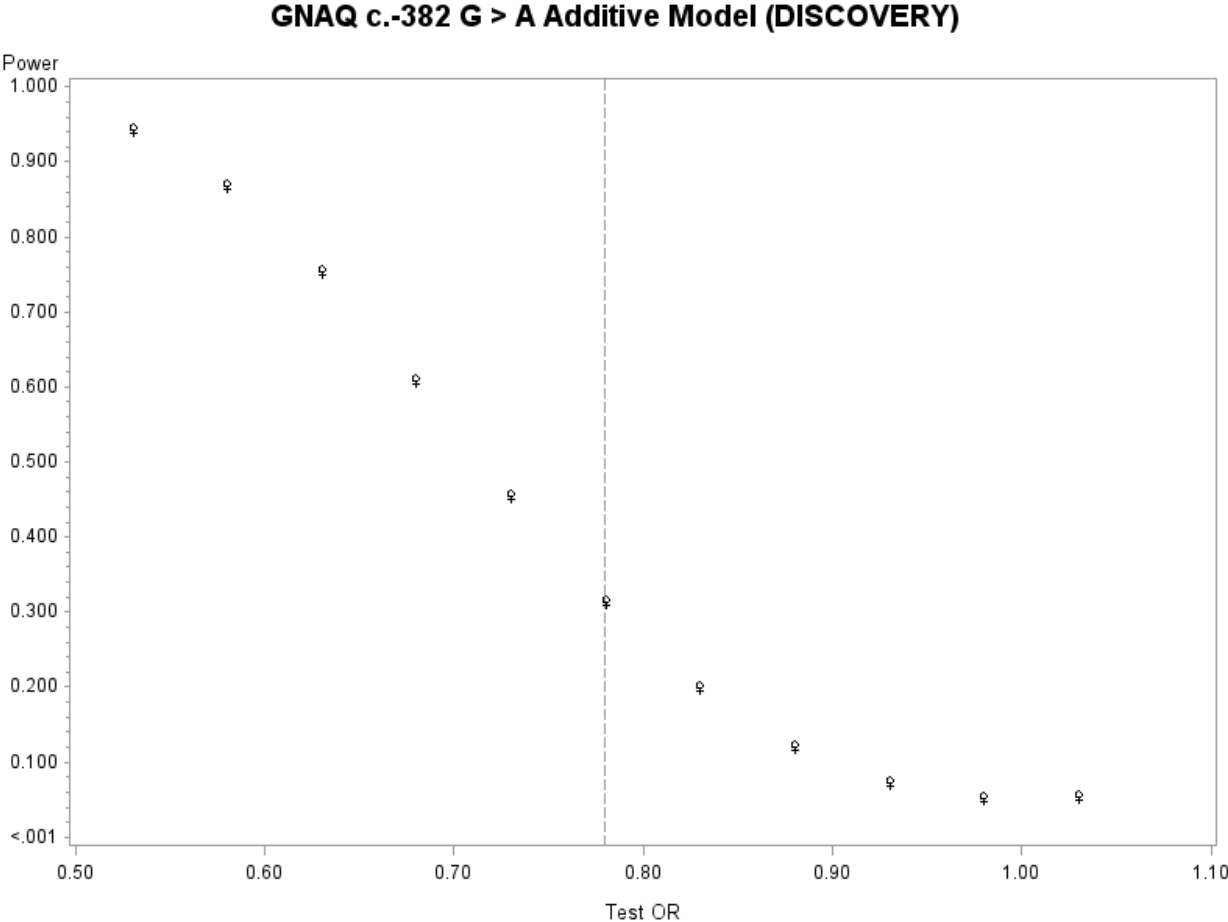


Figure S6. Achieved power of the GNAQ c.-387 G>A SNP in the DISCOVERY study (additive model) over a range of test odds ratios (OR).

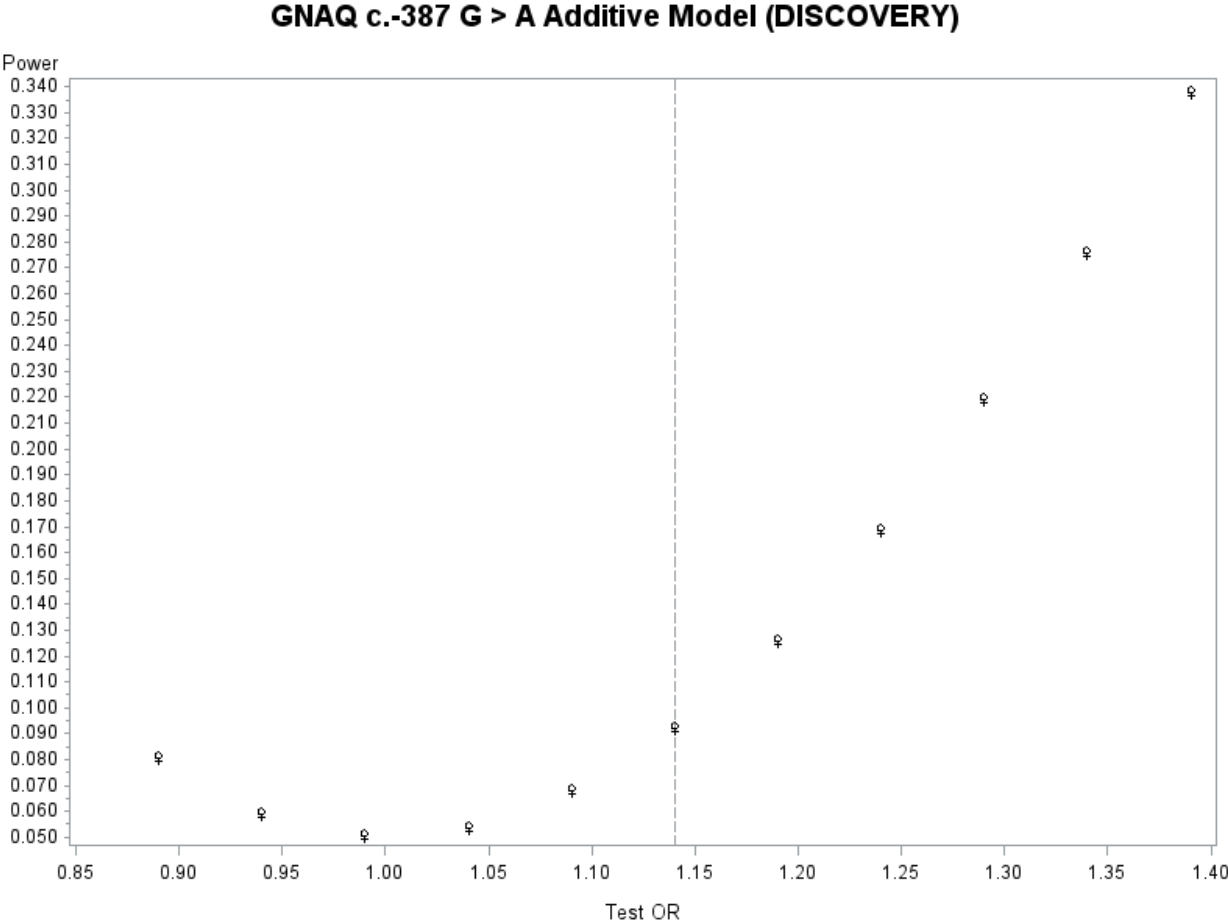


Figure S7. Achieved power of the GNAQ c.-909/-908 GC>TT SNP in the DISCOVERY study (additive model) over a range of test odds ratios (OR).

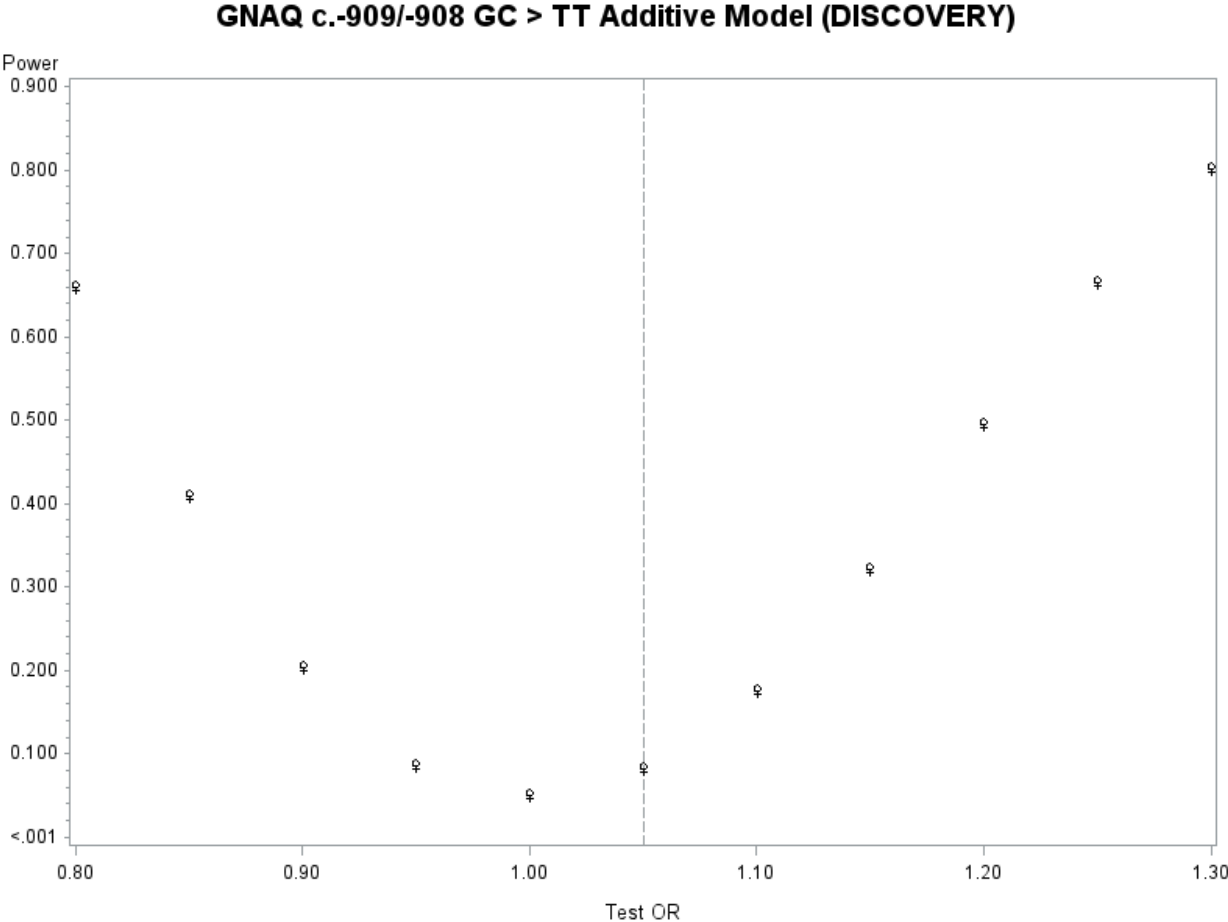


Figure S8. Achieved power of the GNAS c.393 C>T SNP in the Oregon SUDS study (additive model) over a range of test odds ratios (OR).

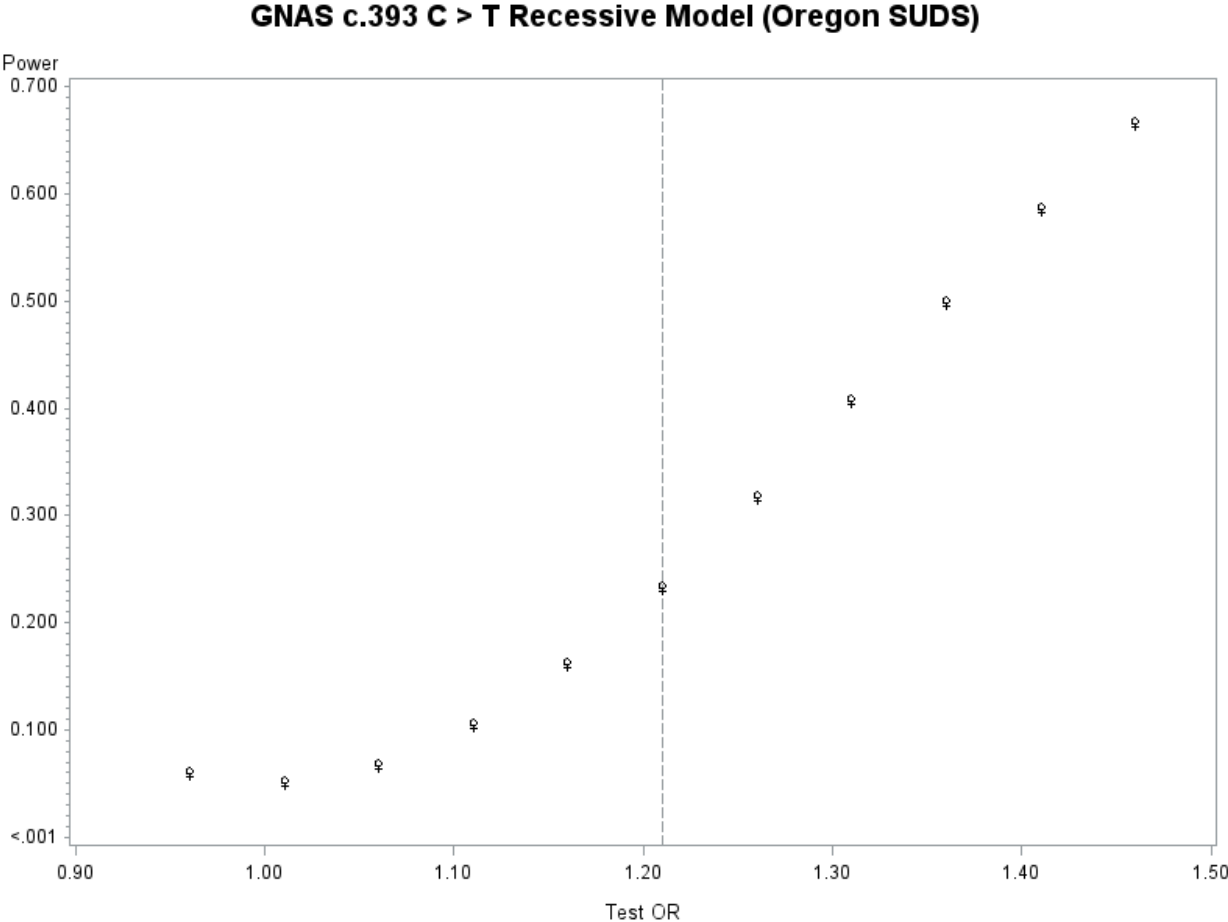
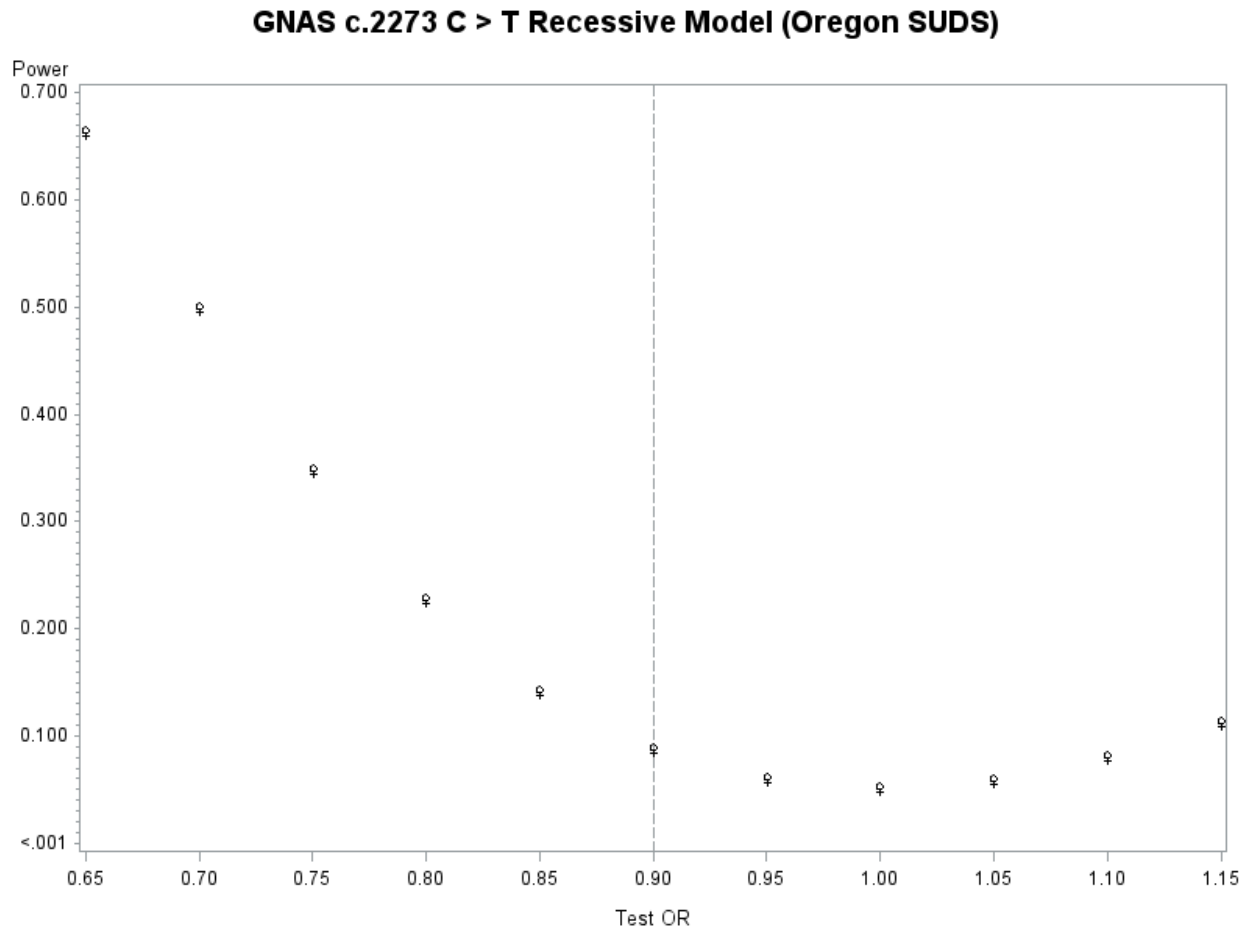


Figure S9. Achieved power of the GNAS c.2273 C>T SNP in the Oregon SUDS study (additive model) over a range of test odds ratios (OR).



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