GNAS Gene Variants Affect β-blocker–related Survival after Coronary Artery Bypass Grafting

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ABSTRACT

Background: Cardiac overexpression of the β-adrenoreceptor (βAR)–coupled stimulatory G-protein subunit Gαs enhances inotropic responses to adrenergic stimulation and improves survival in mice under βAR blockade. The authors recently identified three common haplotypes in the *GNAS* gene encoding Gαs, with the greatest Gαs protein expression and signal transduction in haplotype *3 carriers and less in haplotype *2 and *1 carriers. The authors tested the hypothesis that these *GNAS* variants result in altered mortality in patients after coronary artery bypass graft surgery, particularly in those receiving βAR blockade.

Methods: This prospective analysis included 1,627 European ancestry patients undergoing primary coronary artery bypass graft surgery. Patients were genotyped for two *GNAS* haplotype tagging single-nucleotide polymorphisms defining three major haplotypes. Up to 5-yr all-cause mortality was estimated using a Cox proportional hazard model; hazard ratios and 95% CIs were calculated while adjusting for demographics, clinical covariates, and the new EuroSCORE II.

Results: Univariate analysis revealed haplotype-dependent 5-yr mortality rates (*1/*1: 18.9%, *2/*1: 13.7%, *2/*2: 9.3%, *3/*1: 10.6%, *3/*2: 9.1%, and *3/*3: 9.6%; $P = 0.0006$). After adjustment for other predictors of death, homozygote haplotype *1 carriers showed a doubled risk for death (hazard ratio, 2.2; 95% CI, 1.2 to 3.8; $P = 0.006$). Considering only patients receiving βAR blockers (n = 1,267), the adjusted risk of death even tripled (hazard ratio, 3.0; 95% CI, 1.5 to 6.1; $P = 0.002$).

Conclusions: *GNAS* haplotypes independently associate with an increased risk of death after primary coronary artery bypass graft surgery. These results are most pronounced in patients receiving βAR blockers, strengthening the rationale for personalized treatment, to decrease medication side effects and improve outcomes. **(Anesthesiology 2014; 120:1109-17)**

THE cardiac β-adrenergic receptor (βAR) signal transduction pathway regulates inotropy and chronotropy. βAR stimulation results in activation of the α subunit of the stimulatory G-protein (Gαs), which is coupled to adenylyl cyclase and increases cyclic adenosine monophosphate production. In coronary artery disease–associated ischemic heart failure, chronic stimulation of βARs by increased concentrations of circulating catecholamines evokes βAR desensitization through uncoupling of downstream signaling effectors.[1,2] βAR blocker use results in a restoration of βAR sensitivity and has been associated with slightly increased survival in patients after coronary artery bypass graft (CABG) surgery.[3] However, substantial heterogeneity of individual responses to βAR blocker therapy exists and appear related in part to variation in the adrenergic pathway genes.[4] Yet, results of studies addressing the association of genetic variation in the βAR with cardiac outcomes have been disappointing, and most presumptive associations have not been consistently replicated in later studies.[5–9]

What We Already Know about This Topic

- In animals, overexpression of the G-protein subunit Gαs enhances inotropy from adrenergic stimulation and improves survival in the face of β-adrenoreceptor blockade
- There is genomic variability in the *GNAS* gene for this protein in humans, which may impart risk to surgery and β-blockade

What This Article Tells Us That Is New

- In 1,627 patients undergoing coronary artery bypass graft surgery, those with a *GNAS* haplotype resulting in low expression and function of Gαs showed double the risk of death compared with other haplotypes
- In this high-risk group, those who also received β-blockers showed triple the risk of death

Due to the direct signal transduction from βARs to the G-protein Gαs, Gαs might play a role in βAR blocker–related outcomes in patients undergoing CABG. Supporting

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this, in transgenic mice, overexpression of Gαs using a rat α-myosin heavy chain promoter[10] increases heart rate and cardiac contractility in young transgenic animals but is associated with dilated cardiomyopathy at old age.[11] By contrast, chronic βAR blockade completely prevents the decrease in ejection fraction, cardiac dilation, and premature mortality, characteristic of older transgenic mice overexpressing Gαs.[12] These data indicate significant physiological effects of increased Gαs expression. In humans, however, genetic overexpression experiments are difficult to perform and, therefore, it remains unknown whether altered Gαs expression affects the prognosis of humans with cardiac disease.

Gαs is imprinted in a tissue-specific manner. Although it is expressed primarily from the maternal allele in some select hormone-active tissues, it is biallelically expressed with an almost equal contribution of the maternal and paternal allele in most tissues.[13] Although heterozygous *GNAS* mutations in specific tissues result in different kinds of endocrine disorders,[14] germline variations affecting Gαs expression have not hitherto been identified. By sequencing the human *GNAS* gene, we recently identified two haplotype tagging single-nucleotide polymorphisms (SNPs), G(-1211)A and T2291C, which are located in regulatory regions and form three common haplotypes in the *GNAS* gene.[15,16] Haplotype *3 (A(-1211)/C2291) carriers showed altered transcription factor binding, increased Gαs expression, and enhanced Gαs-stimulated adenylyl cyclase activity. This was associated with a higher stroke volume and cardiac index as well as a lower N-terminal pro-brain natriuretic peptide concentration,[15,16] indicating that germline variants in regulatory regions of the Gαs gene serve as markers to investigate functional and clinical consequences of altered Gαs expression. These observations along with a pilot study of short-term mortality after CABG surgery[17] led us to the hypothesis that *GNAS* variants associate with mortality during the 5 yr after CABG surgery and that this association is modified by the presence or absence of βAR blockade.[11] We further hypothesized that genetic variants would add additional predictive value over the EuroSCORE II surgical risk index, a validated and commonly used method for predicting mortality after CABG surgery.[18,19]

Materials and Methods

Study Sample

Patients aged 20 to 80 yr undergoing nonemergent primary CABG surgery using cardiopulmonary bypass without other concurrent surgery were prospectively enrolled (*http://clinicaltrials.gov/show/NCT01258231*) at two institutions (Brigham and Women's Hospital, Boston, Massachusetts, and Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas) between August 2001 and January 2009. Patients with a preoperative hematocrit of less than 25% or those having received a transfusion of leukocyte-rich blood products within 30 days before surgery were not enrolled. To avoid potential population stratification, analysis was restricted to subjects who self-reported four generations of grandparental European ancestry. Study protocols were approved by the respective Institutional Review Boards, and participants were enrolled after providing informed written consent. At each site, patient demographics, perioperative risk factors, medications, and postoperative outcomes were recorded using study-specific case report forms. Mortality was assessed by accessing hospital records and the Social Security Death Index.† Death status was queried at 5 yr of follow-up or earlier in patients who had not completed 5 yr of follow-up at the time this analysis was performed.

Genotyping

DNA was extracted from leukocytes using standard protocols. Two *GNAS* gene SNPs (Chr. 20): G(-1211)A (rs6123837) and T2291C (rs6026584) and two *ADRB2* gene SNPs (Chr. 5), Arg16Gly (nucleotide 46 A/G, rs1042713), and Gln-27Glu (nucleotide 79 C/G, rs1042714) were genotyped. The method of "slowdown polymerase chain reaction"[20] was used to amplify promoter and intron 1 fragments comprising *GNAS* SNPs as previously described.[16] *GNAS* and *ADRB2* SNPs were genotyped using genotyping assays from Applied Biosystems (Carlsbad, CA). *GNAS* haplotypes were inferred using the Bayesian statistical-based program PHASE, version 2.1,[21] resulting in three common haplotypes (*1, *2, and *3). Individual diplotypes (*i.e.*, haplotype pairs) were constructed from the respective genotypes (see table, Supplemental Digital Content 1, http://links.lww.com/ALN/B35). All SNPs were in Hardy–Weinberg equilibrium.

Statistical Analysis

Data are presented as mean ± SD unless stated otherwise. Univariate comparisons of 5-yr genotype- or diplotype-related mortality were performed using Cox proportional hazard statistics. Kaplan–Meier plots were used to show the relation between genotypes or diplotypes and cumulative mortality. Log-rank tests for trend were performed for genotype- or diplotype-dependent survival using an additive model due to a gene-dose effect. After demonstrating a significant βAR blocker × diplotype interaction on the univariate ($P = 0.018$) as well as the multivariate level ($P = 0.015$), we generated two multivariable (Cox proportional hazards) predictor models of mortality in patients receiving βAR blockers. The first model was generated using clinical and demographic variables that were associated ($P < 0.1$) in univariate analysis as well as covariates being part of the EuroSCORE II with time to death during the first 5 yr after surgery. The second multivariable model incorporated the EuroSCORE II score. Patients who did not die during the 5 yr after CABG were censored at 5 yr or at the last date of follow-up if that was less than 5 yr after surgery.

Proportional hazards assumptions were obtained by computing correlations between time and partial residuals, which are calculated separately for each genetic predictor. Partial residuals were plotted against survival time to test the proportional hazards assumption by adding a smoother to the residual plots.

† Available at: http://searches.rootsweb.ancestry.com/ssdi.html. Accessed December 16, 2013.

In addition, general linear regression analysis was performed for partial residuals against time. A nonzero correlation was regarded as evidence against the proportionality assumption.

This study takes the advantage of an ongoing registry and longitudinally updated data set, CABG Genomics, which has been used for previous analyses of genetic associations. We are aware of potential problems of multiplicity in a data set over time, but adjusting the α error for previous analyses is difficult and has traditionally not been done. Hence, neither attempts were made to adjust the error rate for past or future analyses of these data, nor did we consider multiple genotype predictors.

Data were analyzed using the SPSS software package, version 19.0 (IBM, New York, NY). A two-tailed *P* value of less than 0.05 was considered statistically significant.

Results

Patient characteristics stratified by *GNAS* genotypes are shown in table 1. Whereas only a significant association of T2291C and sex could be detected, no association of *GNAS* genotypes and variables of cardiovascular disease prevalence were identified.

During the up to 5-yr postoperative follow-up period, 10.9% of subjects died. Median follow-up time of surviving

Table 1. Demographic Variables and Statistics as Related to *GNAS* Genotypes

	G(-1211)A				T2291C			
	AA (n = 216)	AG (n = 719)	GG (n = 692)	*P* Value	CC (n = 729)	CT (n = 697)	TT (n = 201)	*P* Value
Demographics								
Sex, N (% male)	173 (80.1)	564 (78.4)	565 (81.6)	0.322	569 (78.1)	578 (82.9)	155 (77.1)	0.039
Age, yr (range)	65 (35–86)	64 (25–89)	65 (35–89)	0.568	65 (25–89)	64 (28–89)	65 (41–89)	0.136
BMI (kg/m^2)	29.4 ± 5.2	29.6 ± 5.7	30.0 ± 5.7	0.318	29.7 ± 5.7	29.6 ± 5.6	30.1 ± 5.9	0.642
Euroscore II	3.6 ± 4.0	3.8 ± 5.3	4.0 ± 5.2	0.578	3.8 ± 5.0	3.8 ± 5.1	4.4 ± 5.4	0.231
Medical history								
Preoperative LVEF, (%)	52 ± 12	51 ± 12	52 ± 11	0.411	52 ± 12	52 ± 12	51 ± 13	0.963
Diabetes mellitus, N (%)	82 (38)	252 (35)	218 (32)	0.151	255 (35)	222 (32)	75 (37)	0.256
Pulmonary disease, N (%)	29 (13)	118 (16)	126 (18)	0.244	110 (15)	123 (18)	40 (20)	0.195
Creatinine concentration, (mg/dl)	1.1 ± 0.4	1.1 ± 0.3	1.1 ± 0.3	0.460	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	0.383
Hematocrit, (%)	41 ± 4	41 ± 4	40 ± 4	0.942	40 ± 4	41 ± 4	40 ± 4	0.382
Hypertension, N (%)	157 (73)	543 (76)	527 (76)	0.583	557 (76)	524 (75)	147 (73)	0.537
Hypercholesterolemia, N (%)	169 (78)	532 (74)	547 (79)	0.068	562 (77)	521 (75)	165 (82)	0.090
Coronary stenosis (>50%) regions, N (%)								
≤2	37 (18)	175 (25)	172 (26)		170 (24)	167 (25)	47 (24)	
3	118 (56)	352 (51)	343 (52)		371 (53)	344 (51)	98 (50)	
≥4	56 (27)	170 (24)	151 (23)	0.147	165 (23)	161 (24)	51 (26)	0.940
Previous myocardial infarction, N (%)	89 (41)	285 (40)	283 (41)	0.860	300 (41)	272 (39)	85 (42)	0.601
NYHA, N (%)								
I	71 (33)	218 (30)	224 (32)		239 (33)	212 (30)	62 (31)	
II	86 (40)	304 (42)	291 (42)		287 (39)	304 (44)	90 (45)	
III	46 (21)	164 (23)	142 (21)		167 (23)	148 (21)	37 (18)	
IV	13 (6)	33 (5)	35 (5)	0.876	36 (5)	33 (5)	12 (6)	0.580
Medications: preoperative, N (%)								
β-Blocker	176 (81)	563 (78)	528 (76)	0.259	583 (80)	533 (77)	151 (75)	0.170
Aspirin	171 (79)	563 (79)	533 (77)	0.746	585 (81)	528 (76)	154 (77)	0.093
HMG-CoA reductase inhibitor	168 (78)	537 (75)	515 (75)	0.655	562 (78)	503 (73)	155 (78)	0.057
Surgery								
No. of grafts, N (%)								
≤2	26 (12)	128 (18)	115 (17)		113 (16)	122 (18)	34 (17)	
3	93 (43)	338 (47)	316 (46)		342 (47)	302 (43)	102 (51)	
≥4	97 (45)	253 (35)	261 (38)	0.088	274 (38)	273 (39)	64 (32)	0.237
CPB duration, min	101 ± 43	100 ± 45	98 ± 41	0.742	99 ± 41	100 ± 46	97 ± 37	0.695
Aortic cross-clamp duration, min	75 ± 35	74 ± 35	72 ± 35	0.458	74 ± 34	74 ± 36	70 ± 35	0.339
Intraoperative β-blocker administration, N (%)	35 (16)	124 (17)	100 (15)	0.350	119 (16)	112 (16)	28 (14)	0.709
Intraoperative IABP, N (%)	5 (2)	12 (2)	15 (2)	0.737	13 (2)	10 (1)	9 (4)	0.098

Data are shown as percentages for dichotomous variables and as means ± SD for continuous variables. Diabetes mellitus was defined as insulin dependent or noninsulin dependent. *P* values refer to univariate Cox regression analysis.

BMI = body mass index; CPB = cardiopulmonary bypass; HMG = 3-hydroxy-3-methyl-glutaryl-CoA reductase; IABP = intraaortic ballon pump; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

patients was 5.0 yr (range, 2.4 to 5.0 yr). Univariate analysis demonstrated that *GNAS* variants, but not *ADRB2* variants, had a significant association with all-cause mortality (table 2).

GNAS variants showed a significant association with mortality for the G(-1211)A variant (GG: 13.2%, AG: 9.8%, and AA: 9.6%; $P = 0.012$; fig. 1A) and the T2291C variant (TT: 18.9%, CT: 12.1%, and CC: 9.2%; $P = 0.0003$; fig. 1B). Estimation of diplotypes confirmed these results with 5-yr mortality for diplotype *1/*1: 18.9%, *2/*1: 13.7%, *2/*2: 9.3%, *3/*1: 10.6%, *3/*2: 9.1%, and *3/*3: 9.6% (overall $P = 0.0006$; fig. 1C). Consideration of clinical covariates revealed a hazard ratio of 2.2 for homozygous *1 *versus* *3 (95% CI, 1.2 to 3.8; $P = 0.006$).

We next examined *GNAS* genotype association with 5-yr mortality stratified by βAR blocker use. When we analyzed patients without βAR blocker therapy, we observed no genotype effect on 5-yr mortality (fig. 1, D–F). In contrast, there was a strong association with mortality in patients receiving βAR blockers. Five-year mortality was associated with genotypes of the *GNAS* variants G(-1211)A (GG: 14.1%, AG: 9.2%, and AA: 6.9%; $P = 0.0014$; fig. 1G), T2291C (TT: 19.4%, CT: 11.5%, and CC: 8.2%; $P = 0.0002$; fig. 1H), and estimated diplotypes (*1/*1: 19.4%, *2/*1: 13.5%, *2/*2: 9.2%, *3/*1: 9.7%, *3/*2: 8.6%, and *3/*3: 6.9%; $P < 0.0001$; fig. 1I). In multivariable analysis, after verifying proportional hazard assumptions, these findings remained present after accounting for clinical factors previously found to be associated with mortality and revealed increased mortality for *2/*1 and *1/*1 patients compared with that for homozygous *3 patients (table 3). We also estimated the additional value of *GNAS* haplotype data on the widely used EuroSCORE II to predict survival after CABG surgery. As expected, we not only observed strong predictive value of the EuroSCORE II but also observed that *GNAS* haplotypes were prognostic factors for 5-yr survival, independent of the EuroSCORE II. Although the EuroSCORE II resulted in a hazard ratio of 1.07 (95% CI, 1.06 to 1.09; $P < 0.001$), patients homozygous for the *1 *GNAS* haplotype had a more than three-fold increased 5-yr mortality compared with that for patients homozygous for *3 (hazard ratio, 3.14; 95% CI, 1.57 to 6.31; $P = 0.001$).

In this cohort, βAR blocker use *per se* was not associated with overall survival (fig. 2A). However, stratification by *GNAS* haplotypes demonstrated marked differences in the effect of βAR blocker use on survival. Significantly better survival was seen for patients using βAR blockers and being *3 positive (91.3%). In contrast, reduced survival was observed in patients not possessing the *3 haplotype irrespective of βAR blocker therapy (85.6 to 85.9%) or in haplotype *3 positive patients without βAR blocker therapy (85.8%; fig. 2B; $P = 0.0085$).

Table 2. Genotype-specific Survival

	Alive (N = 1,450)	Died (N = 177)	*P* Value
Genotype, N (%)			
ADRB2 Arg16Gly (n = 1,549)			
Gly/Gly	562 (40)	62 (39)	
Arg/Gly	635 (46)	73 (46)	
Arg/Arg	194 (14)	23 (15)	0.94
ADRB2 Glu27Gln (n = 1,550)			
Gln/Gln	483 (35)	47 (30)	
Glu/Gln	639 (46)	87 (55)	
Glu/Glu	269 (19)	25 (16)	0.13
GNAS G(-1211)A			
GG	600 (41)	92 (52)	
AG	653 (45)	66 (37)	
AA	197 (14)	19 (11)	0.025
GNAS T2291C			
TT	165 (11)	36 (20)	
CT	619 (43)	78 (44)	
CC	666 (46)	63 (36)	0.001
GNAS diplotype			
*1/*1	165 (11)	36 (20)	
*2/*1	280 (19)	41 (23)	
*2/*2	155 (11)	15 (9)	
*3/*1	339 (23)	37 (21)	
*3/*2	314 (22)	29 (16)	
*3/*3	197 (14)	19 (11)	0.008

Data are shown as numbers with percentages in brackets. *P* values refer to univariate Cox regression analysis.

Discussion

Our data show that *GNAS* haplotypes associate with significantly different rates of death during the 5 yr after CABG surgery. *GNAS* haplotype *1 or *2 carriers show an increased risk of death compared with the risk by *3 haplotypes. Importantly, this effect is more pronounced in patients receiving βAR blockers and is independent of other traditional risk factors.

These findings also suggest that routine use of βAR blockers in *GNAS* haplotype *3 negative patients undergoing cardiac surgery may not provide significant long-term mortality benefit. Furthermore, we observed that the addition of *GNAS* diplotypes to the well-validated EuroSCORE II provides additional predictive value for long-term postoperative mortality. The frequency of homozygosity of the *1 haplotype, similar to that observed in a different smaller CABG cohort,[17] establishes the *GNAS* haplotype as a common disease-linked haplotype with a novel association to a long-term outcome, whereas an association to cardiovascular disease prevalence has not been detected.

Experiments from transgenic mice indicate the importance of the Gαs protein in the maintenance and augmentation of cardiac function.[11,12,22] We recently identified three common haplotypes in the *GNAS* gene encoding Gαs[16] with haplotype-specific differences in *GNAS* promoter activity.[16,17] In human myocardium, we could demonstrate the greatest Gαs protein expression and signal transduction in haplotype *3 carriers, followed by haplotypes *2 and *1.[17] Interestingly, diminished Gαs expression in haplotype

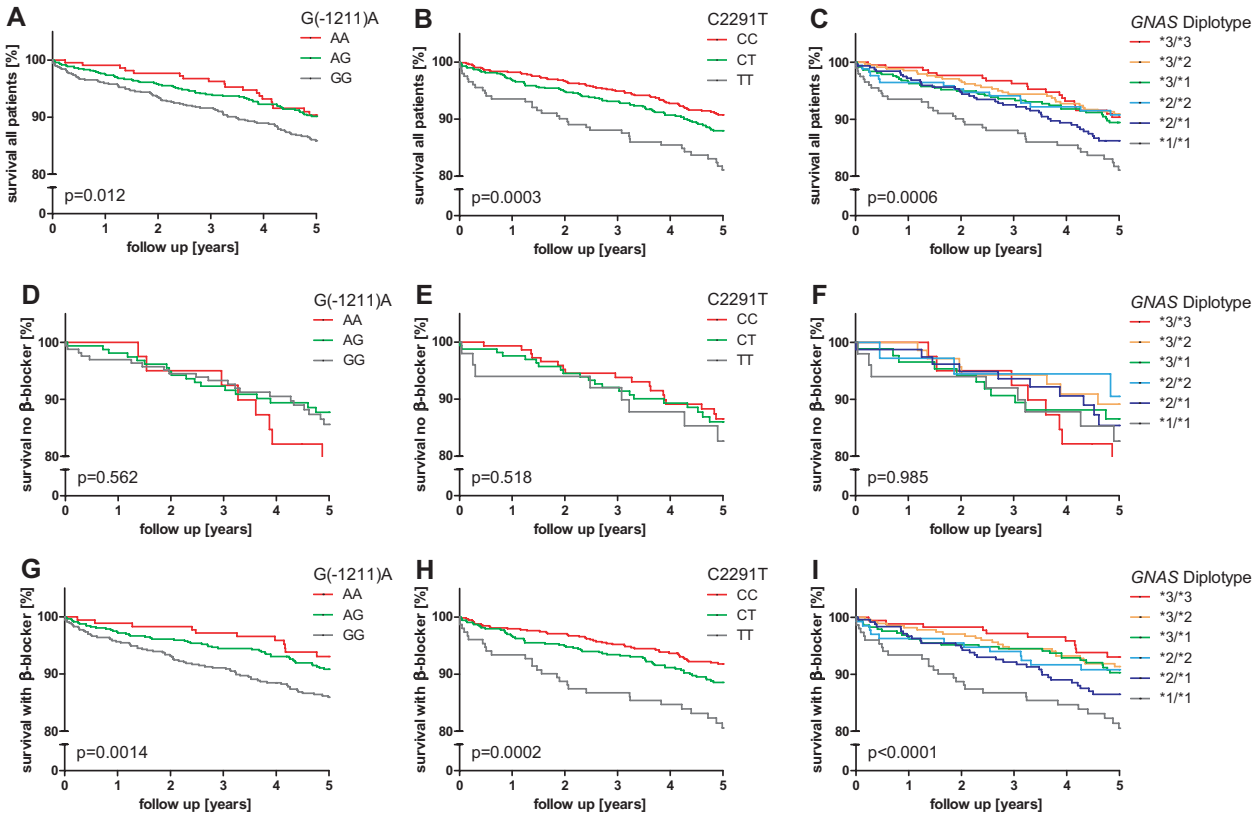

Fig. 1. Kaplan–Meier plots for survival dependent on *GNAS* polymorphisms and respective diplotypes. (*A–C*) Survival analysis for all patients (n = 1,627). (*D–F*) Survival analysis for patients without β-adrenergic receptor blockade (n = 360). (*G–I*) Survival analysis for patients under β-adrenergic receptor blockade (n = 1,267); *P* values represent log-rank test for trend.

*1 carriers was associated with a higher βAR density,[16] a feature pathognomonic of βAR up-regulation.[23] However, it has to keep in mind that those experiments were carried out in human atrial tissue and it has been shown that there are differences in the Gαs expression levels between cardiac atria and ventricular myocardium.[24]

Experiments with transgenic mice overexpressing Gαs showed increased sensitivity to catecholamines as a result of enhanced βAR coupling to various effector pathways, specifically adenylyl cyclase and L-type calcium channels.[10,11,25] However, the effects of chronically enhanced β-adrenergic signalling over the lifespan of the animal may be deleterious, particularly in the face of ineffective βAR-desensitization mechanisms, as these mice developed cardiomyopathy at older age.[26] These experiments in transgenic animals are in contrast to our findings, where we identified a 30% increase of Gαs expression in humans in homozygous haplotype *3 carriers,[16] which was also associated with a survival benefit in patients with enhanced Gαs expression (haplotype *3). In transgenic mice, however, Gαs expression was up-regulated more than three-fold compared with that in wildtype mice.[22] Therefore, we hypothesize that higher Gαs expression in homozygous haplotype *3 carriers might result in a sensitization of βAR, resulting in a greater number of βAR in the "high-affinity state" resulting in a survival benefit.[10]

Our findings showing a survival benefit from βAR blockade in patients with haplotype *3 (increased Gαs expression), compared with other patients (fig. 2B), are in agreement with chronic βAR blockade in transgenic mice overexpressing Gαs resulting in protection from evoked heart failure.[10–12,22,25] We, therefore, speculate that resensitization of βAR by βAR blockade in patients with increased Gαs expression results in a long-term patient benefit.

Another issue that has to be considered is the potential effect of altered Gαs expression in ischemia–reperfusion damage during CABG surgery. Many pathological processes contribute to ischemia- and reperfusion-associated heart injury. For example, ischemia during extracorporal circulation is associated with decreased adenylate cyclase activity and intracellular cyclic adenosine monophosphate concentrations and may result in increased vascular permeability, endothelial cell inflammation, an imbalance between vasodilating and vasoconstricting factors, and the activation of coagulation and the complement system.[27] Therefore, increased cardiac Gαs concentration with increased intracellular cyclic adenosine monophosphate levels during that period could evoke beneficial effects regarding attenuated reperfusion injury and inflammatory response.

We hypothesized that enhanced Gαs-mediated signal transduction might yield a more beneficial outcome after

Table 3. Multivariable (Cox Proportional Hazards) Predictor Model of Mortality in Patients on βAR Blockade (n = 1,267)

Predictor	HR	95% CI Lower	95% CI Upper	*P* Value
Sex				
Female	1*			
Male	1.27	0.81	2.00	0.308
Age (yr)	1.05	1.03	1.07	<0.001
Preoperative LVEF (%)	0.97	0.96	0.99	<0.001
Diabetes mellitus				
No	1*			
Yes	1.49	1.04	2.13	0.030
Pulmonary disease				
No	1*			
Yes	1.49	0.96	2.18	0.075
Creatinine concentration (mg/dl)	1.68	1.10	2.56	0.015
Hematocrit (%)	0.95	0.90	0.99	0.014
NYHA	1.27	1.04	1.56	0.019
Number of grafts	0.71	0.55	0.92	0.008
GNAS diplotype				
*3/*3	1*			
*3/*2	1.27	0.61	2.62	0.521
*3/*1	1.51	0.74	3.07	0.252
*2/*2	1.33	0.58	3.04	0.502
*2/*1	2.27	1.14	4.53	0.020
*1/*1	3.02	1.50	6.11	0.002

Clinical and demographic variables were selected based on association ($P < 0.1$) in univariate analysis or when part of the EuroSCORE II.

* Reference.

βAR = β-adrenoreceptor; HR = hazard ratio; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

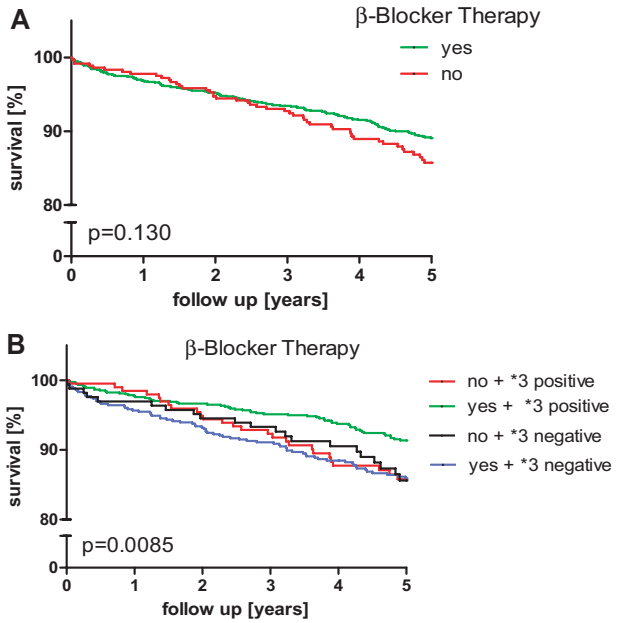

Fig. 2. Kaplan–Meier survival analysis stratified by β-adrenoreceptor (βAR) blocker therapy. (*A*) Patients on βAR blockade (n = 1,267) and patients without β-adrenergic receptor blockade (n = 360). (*B*) Analyses for haplotype *3 positive patients with (n = 739) and without β-adrenergic receptor (n = 196) and of haplotype negative patients with (n = 528) and without β-adrenergic receptor blockade (n = 164). *P* values represent log-rank test for comparison of all curves.

CABG, particularly under βAR blockade, and this hypothesis was tested, first in a pilot study,[17] and now in a large multicenter cohort of patients. In the pilot study including 185 patients under chronic βAR blockade, Gαs expression was diminished in homozygous haplotype *1 patients and this was associated with an increased risk of death compared with the risk in homozygous haplotype *3 carriers, with an independent hazard ratio of 3.1.[17] The current study in 1,627 patients with a 5-yr follow-up now extends these pilot data in a new cohort of well-characterized patients and confirms the primary hypothesis. In a multivariable analysis, homozygous *1 patients had a doubled risk of death compared with that of homozygous *3 patients and stratification by the presence or absence of βAR blockade tripled this risk, independently of conventional risk factors.

From a pharmacogenetic point of view, our observations are important as βAR blockers are the cornerstone therapy for patients with coronary artery disease, and the use of βAR blockers in the perioperative period remains a topic of intense research.[28] Although the consistent efficacy of βAR blockers has been questioned, variability of effects was suggested to relate in part to genetic heterogeneity.[29,30] On the basis of our observations in the current study, future studies of βAR blockers should include accounting for genotype structure of genes in the βAR pathway. Importantly, this study questions the value of routine use of βAR blockers in all patients undergoing cardiac surgery, an issue which was already raised during the early termination of the PeriOperative ISchemic Evaluation trial,[31] in which patients who received βAR blockers had an increased risk of strokes. However, the investigational βAR blocker bucindolol, which in the Beta-Blocker Evaluation of Survival Trial trial[32] did not show an overall survival benefit compared with placebo, was associated with fewer adverse events in patients with a βAR genetic variant.[33] Thus, without pharmacogenetic data, there exists a tradeoff between the benefit of a medication in a minority of patients and the possibly associated risks and costs in the majority of patients.

Finally, despite improved survival in a pilot study of patients with various Gly16Gly genotypes undergoing CABG surgery,[17] we did not find an association of *ADRB2* polymorphisms with long-term survival in the current study. Therefore, we cannot draw a definitive conclusion of whether *ADRB2* genotypes influence outcome after CABG surgery. However, a recent study[34] of patients with acute coronary syndrome does not support a significant impact of *ADRB2* genotypes on outcome in American patients of Caucasian ethnicity.

Limitations

Our study should be interpreted in the context of some potential limitations. The study of this longitudinal observational

cohort was not designed to investigate βAR blocker–specific effects on long-term survival. Due to a high level of adherence to βAR blocker guideline recommendations, only a small fraction of subjects were left untreated with βAR antagonists. This precluded a formal analysis to investigate the statistical interaction of genotype with βAR antagonist treatment. Nevertheless, we identified genetic subgroups with worse outcomes despite βAR antagonist treatment, in whom more aggressive interventions to improve survival might be warranted. In addition, we had no information on the various βAR blockers used in this cohort and thus cannot ascertain agent-specific effects, if present. Moreover, we were unable to account for changes in medications during follow-up time. Furthermore, classifying patients by discharge medication status is a well-recognized and often-used approach because most patients remain on their discharge regimen after hospital stay.[35]

This is an analysis of an ongoing study in a longitudinal cohort for which we have previously reported associations between genetic variants and atrial fibrillation,[36] myocardial injury,[37–39] ventricular dysfunction,[40,41] inflammation,[42] and up to 5-yr postoperative all-cause mortality.[41,43–45] The current study's assessment of *GNAS* variants associated with 5-yr mortality did not adjust for previous analyses of these other gene-association studies in the CABG Genomics Program cohort. Finally, it may be speculated that one or several yet unidentified functional SNPs in adrenergic or other pathway genes such as the A2B adenosine receptor or the β1-receptor could influence our results.[6,46] However, due to our hypothesis-driven approach following a pilot study,[17] analysis of those genes was not the aim of our current study but should be considered with future studies. Moreover, besides the β-adrenergic signaling pathway, Gαs is also activated by several other G-protein–coupled receptors,for example, including the serotonin and adenosine receptors.[47] It is, therefore, conceivable that it is not solely the β-receptor–mediated signal transduction pathway that is responsible for the observed effects but also cumulative or single effects mediated *via* other Gαs-mediated signaling pathways.

In conclusion, *GNAS* haplotypes independently associate with an increased risk of death after primary CABG surgery. These results are most pronounced in patients receiving βAR blockers and strengthen the rationale for personalized treatment to decrease medication side effects and improve outcomes.

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Competing Interests

The authors declare no competing interests.

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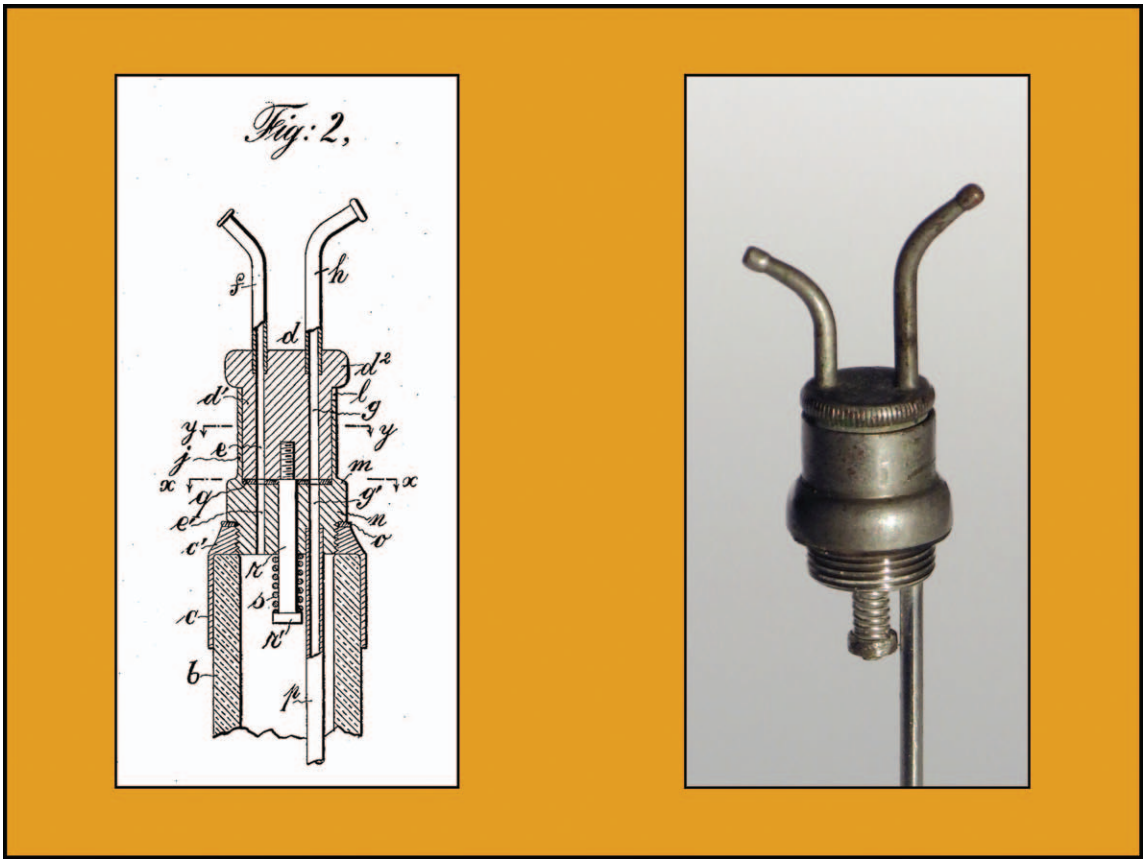
The Wachter Chloroform Dropper



An American citizen residing in Jersey City, New Jersey, Frederick Wachter filed on Columbus Day of 1905 for a U.S. patent on his "Chloroform-Dropper." On the right side of both images, an air-venting tube *h* extends down through the stopper and deep inside the drop bottle as tube *p*. A central screw turns through 90 degrees from *s*[*hut*] to *o*[*pen*]. When fully open, with bent tubes pointed downward, chloroform can pass through the left tubing on both images, from *e* to *f*. Granted on June 12, 1906, [U.S.] "PAT No 823233" was inscribed across the dropper top. One of the few examples of dropper bottle technology not designed in Europe, the Wachter Chloroform Dropper was designed, as Europeans will gleefully note, by a German-American who was born in Baden, Germany. (Copyright © the American Society of Anesthesiologists, Inc.)

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