GNAQ TT(-695/-694)GC Polymorphism Is Associated with Increased Gq Expression, Vascular Reactivity, and Myocardial Injury after Coronary Artery Bypass Surgery

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ABSTRACT

Background: Angiotensin II receptor type 1–mediated activation of the α-subunit of the heterotrimeric Gq protein evokes increased vasoconstriction and may promote hypertrophy-induced myocardial damage. The authors recently identified a TT(-695/-694)GC polymorphism in the human Gq promoter, the GC allele being associated with an increased prevalence of cardiac hypertrophy. In this article, the authors tested whether the TT(-695/-694)GC polymorphism is associated with differences in (1) myocardial Gq protein expression, (2) vascular reactivity, and (3) myocardial damage after coronary artery bypass grafting.

Methods: Gq protein expression was measured in right atrial muscle from 55 patients undergoing coronary artery bypass grafting as were skin perfusion changes (n = 18; laser Doppler imaging), saphenous vein ring vascular reactivity (n = 50, organ bath) in response to angiotensin II, and myocardial damage (227 patients undergoing coronary artery bypass grafting), as assessed by postoperative cardiac troponin I concentration.

Results: Myocardial Gq expression was greater in GC/GC genotypes (GC/GC *vs.* TT/TT: 1.27-fold change; $P = 0.006$). Skin perfusion after intradermal angiotensin II injection decreased only in GC/GC genotypes ($P = 0.0002$). Saphenous vein rings exposed to increasing angiotensin II concentrations showed an almost doubled maximum contraction in GC/GC compared with individuals with the TT/TT genotype ($P = 0.022$). In patients undergoing coronary artery bypass grafting, baseline cardiac ejection fraction was different (GC/GC: 55 ± 13%; GC/TT: 54 ± 14%; TT/TT: 48 ± 15%; $P = 0.037$) and postoperative peak cardiac troponin I was greater in patients with the GC/GC (11.5 ± 13.8 ng/ml) than in patients with the GC/TT (9.2 ± 9.2 ng/ml) or patients with the TT/TT genotype (6.6 ± 4.8 ng/ml, $P = 0.015$).

Conclusions: The GC/GC genotype of the TT(-695/-694)GC polymorphism is associated with increased Gq protein expression, augmented angiotensin II receptor type 1–related vasoconstriction, and increased myocardial injury after coronary artery bypass grafting, highlighting the impact of Gq genotype variation. **(Anesthesiology 2017; 127:70-7)**

LEFT ventricular hypertrophy is an adaptational response to an increased load that involves increased protein synthesis and cardiomyocyte size. However, with pathologic conditions such as hypertension or after myocardial infarction, maladaptive cardiac hypertrophy can result in tissue fibrosis and is associated with a greater mortality due to heart failure and arrhythmia.[1,2] Moreover, left ventricular hypertrophy is associated with changes in the density, structure, and coronary vasodilator capacity so that the cross-sectional diameter of endomyocardial capillaries and coronary reserve are decreased even in the absence of detectable coronary atherosclerosis.[3,4]

Interestingly, activated mutants of the G-protein α-subunit Gq promote myocardial hypertrophy.[5] In line with these studies, knockout of Gq or the functionally similar G protein G11 in cardiomyocytes abolished pressure overload–induced myocardial hypertrophy.[6] Activation of the Gq pathway *via* angiotensin II and the angiotensin II receptor type 1[7] results in activation of phospholipase c beta, which hydrolyses the plasma membrane phosphatidylinositol

What We Already Know about This Topic

- Previous studies have demonstrated angiotensin II receptor type 1–mediated activation of the α-subunit of the heterotrimeric Gq protein evokes increased vasoconstriction and may promote hypertrophy-induced myocardial damage
- This study determined whether a TT(-695/-694)GC polymorphism in the human Gq promoter is associated with differences in (1) myocardial Gq protein expression, (2) vascular reactivity, and (3) myocardial damage after coronary artery bypass grafting

What This Article Tells Us That Is New

- The GC/GC genotype of the TT(-695/-694)GC polymorphism is associated with increased Gq protein expression, augmented angiotensin II receptor type 1–related vasoconstriction, and increased myocardial injury after coronary artery bypass grafting

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4,5-bisphosphate to generate the second messengers inositol 1,4,5-trisphosphate, a regulator of the intracellular calcium response, and diacylglycerol, an activator of protein kinase C subtypes, thereby evoking an diverse array of cellular responses, *e.g.*, vasoconstriction and cardiac hypertrophy.[8]

In patients undergoing coronary artery bypass grafting, recovery of contractile function after reperfusion is depressed, and the serum concentration of cardiac troponin I (cTnI), a marker of myocardial damage, is increased to a much greater extent in hypertrophied hearts subjected to global ischemia. This finding suggests greater susceptibility to ischemia–reperfusion injury of hypertrophied hearts, especially in patients with coronary artery disease in whom coronary flow reserve is diminished independently of stenosis severity.[9–11]

We previously characterized the promoter of the *GNAQ* gene encoding the Gq subunit of heterotrimeric G proteins and identified a novel functional TT(-695/-694) GC promoter polymorphism resulting in increased gene transcription.[12] Allele frequencies are different between ethnic groups, with a GC allele frequency of 0.52 in white,[12] 0.67 in African American,[13] and 0.81 in Chinese populations.[14]

Accordingly, we now tested in an *a priori* analysis whether the TT(-695/-694)GC polymorphism is associated with differences in (1) myocardial muscle Gq protein expression, (2) vascular reactivity, and (3) myocardial damage after coronary artery bypass grafting.

Materials and Methods

Gq Expression Analysis

Following ethics committee approval and written informed consent from all patients, right atrial appendages were obtained as part a former study investigating Gq mRNA expression before cardiopulmonary bypass in patients undergoing coronary artery bypass grafting between 2006 and 2007.[12] Immediately after sampling, specimens were transferred into carbogenated Tyrode solution, quickly frozen in liquid nitrogen, and stored at –80°C. After the collection of appendages from a sufficient number of patients, tissues were split in liquid nitrogen, and the remaining samples were stored in liquid nitrogen for membrane preparations (n = 55). Membranes were prepared as follows: 100 mg tissue was washed in phosphate-buffered saline, minced with a scalpel, and homogenized in 1 ml ice-cold buffer H (300 mM sucrose, 25 mM HEPES; pH 7 with Tris) and a complete Protease Inhibitor Cocktail (Roche Applied Sciences, Germany). Samples were centrifuged at 1,000*g* for 20 min. The supernatant was centrifuged at 80,000*g* (Beckman, Fullerton, USA). After the supernatant was discarded, the pellet was resuspended in 40 µl buffer H. Membrane proteins (30 µg protein per lane) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by Western Blot analysis with an anti-$Gq_{/11}$ antibody (Upstate, USA) or, after stripping the blot, anti-Actin (Santa Cruz Biotechnology, USA). Films were scanned and signals were quantified by densitometry (NIH Image, Scion, USA). To compare Gq expression between different genotypes, the average signal intensity was multiplied by the number of pixels in that area and corrected for the Gβ signal present in the same lane (calculated the same way).

Blood Sampling for Cardiac Biomarker Analysis and DNA Genotyping

Venous blood samples were drawn from each patient the day before coronary artery bypass grafting surgery and postoperatively at 6, 12, 24, 48, 72, and 96 h and were analyzed for cTnI in an accredited laboratory with a specific two-side immunoassay (Dimension Flex; Dade Behring GmbH, Germany). The detection range for cTnI was 0.04 to 40 ng/ml, requiring further dilutions if necessary. The assay's reference interval was 0.00 to 0.05 ng/ml. A cTnI value greater than 0.1 ng/ml was considered abnormal. All laboratory measurements were made without knowledge of *GNAQ* genotypes.

Genomic DNA was extracted from whole blood via the use of standard techniques. A 368-bp polymerase chain reaction fragment was amplified with primer Gq_Se4 (5′-CCCCCTGCCCCGATTGCCA-3′) and Gq_AS4 (5′-GGGTCTGGCCCCGACTTCG-3′), as described previously,[12] with a slowdown polymerase chain reaction technique including 5% dimethyl sulfoxide.[15] Genotypes of the TT(-695/-694)GC polymorphism were determined by restriction with *Nae*I (New England Biolabs, Germany), separation on a 2.5% agarose gel, and visualization under ultraviolet illumination.

Assessment of Skin Microcirculation by Laser Doppler Imaging

Skin microcirculation experiments had been performed previously as part of a study addressing the effects of angiotensin II receptor type 1 receptor antagonism on various vasoconstrictors (data collection between 2004 and 2005)[16] and were analyzed retrospectively to assess the influence of the Gq TT(-695/-694)GC polymorphism. Eighteen white male volunteers (age: 29 ± 4 yr, mean ± SD; *GNAQ* genotype: n = 5 GC/GC, n = 7 GC/TT, n = 6 TT/TT) were studied. All participants were nonsmokers and healthy on the basis of their medical history, physical examination, electrocardiogram results, and routine clinical chemistry screening, and they had a body mass index of 25 kg/m^2 or less. Each volunteer provided written informed consent, and the study was approved by the University of Duisburg-Essen Medical School Ethics Committee (Duisburg, Germany).

A laser Doppler image scanner (Moor LDI; Moor Instruments Ltd., UK) was used to assess skin perfusion, as described previously.[16] To summarize in brief, before intradermal injections, the volar surface of the arm was scanned to assess resting blood flow at each injection site. Then, 0.01 ml saline was injected intradermally followed by angiotensin II

(10^{-16} and 10^{-14} mol/0.01 ml) or a second injection of saline. The double-injection technique has been applied in several studies and shows a high interday reproducibility.[16]

Measurements of Vascular Function In Vitro

Following written informed consent and local ethics committee approval, saphenous vein remnants were obtained during coronary artery bypass grafting between 2006 and 2007 from 50 white patients without venous pathology immediately after the last coronary anastomosis had been completed. Saphenous vein remnants were preserved in oxygenated modified Krebs–Henseleit solution (NaCl 118 mM, KCl 4.69 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.04 mM, $NaHCO_3$ 25 mM, D-glucose 11.1 mM, and HEPES 21.8 mM, pH 7.40) until use. Each piece of saphenous vein was cut into four rings of approximately 5 mm width. The rings were mounted between two L-shaped, stainless-steel hooks in organ baths filled with 10 mL oxygenated Krebs–Henseleit solution of 37°C (pH 7.4). Each preparation was secured to an isometric force transducer (FMI, Germany) via a silk thread, and force was recorded with a dedicated computer system (VitroDat; FMI). Each ring was subjected to a pretension of 10 mN, which was maintained throughout the experiment. After an equilibration period of 60 min in the organ bath, the rings were primed and tested for viability by exposing them twice to KCl (final concentration: 40 mM). Cumulative concentration–response curves were then constructed for angiotensin II (10^{-9} to 10^{-5} M; Sigma, USA) with 1-h intervals. The vasoconstrictive responses to different angiotensin II concentrations were calculated as a percentage of the maximum KCl-induced contraction.

Assessment of GNAQ *Polymorphism-related Myocardial Damage after Coronary Artery Bypass Grafting*

Extending another study[17] following approval by the University of Duisburg-Essen medical faculty's ethics committee and informed written consent, we analyzed in a genotype-dependent manner myocardial injury by using cTnI and included 227 white patients between 2007 and 2013 with single- or multivessel coronary artery disease. Patients were assessed after recruitment on the day before coronary artery bypass grafting. Of 268 patients screened initially, 15 refused to participate, 12 eventually underwent combined bypass and cardiac valve repair surgery, and another 14 patients were excluded due to missing data or DNA. None of the patients underwent previous cardiac surgery, and all clinical, laboratory, and angiographic data were obtained from the patients' medical records.

For coronary artery bypass grafting, anesthesia was induced with etomidate (0.3 mg/kg), sufentanil (1 μg/kg), and rocuronium (0.6 mg/kg) and maintained by the administration of isoflurane (end-tidal concentration: 0.6 to 1.0%) and sufentanil (1 to 4 μg/kg), as required. During cardiopulmonary bypass, isoflurane was given *via* a vaporizer connected to the oxygenator's gas supply. Coronary artery bypass grafting was performed *via* a midline sternotomy with moderate hypothermia, aortic cross-clamping, and cardioplegia by Bretschneider solution. The primary endpoint was myocardial injury as assessed by serial cTnI serum concentrations more than 96 h after surgery.

Statistical Analyses

The *GNAQ* polymorphism was tested for conformation with Hardy–Weinberg expectations, and no evidence for a deviation was detected. Descriptive statistics are summarized for categorical variables as frequencies (%) and compared between groups by use of the Fisher exact test. Continuous variables are expressed as means ± SD and were compared between groups with ANOVA. All statistical analyses were two-tailed and performed with SPSS, version 22.0 (SPSS, USA). Because no data regarding linear endpoints (cTnI) with *GNAQ* genotypes are available, an *a priori* power analysis was not possible. Study sample sizes were therefore used based on previous experiences showing a 1.5-fold increased genotype-related Gq mRNA expression and intracellular signal transduction in GC/GC genotypes compared with TT/TT genotypes.[12]

Data from the laser Doppler scanner were analyzed offline after the completion of each experiment with Moor Software V.3.01 (Moor Instruments Ltd.). To assess the net effects of angiotensin II, the values for resting blood flow and saline at each injection site were subtracted from the values obtained for the agonists. All values were presented as mean changes of perfusion units ± SD. Vascular responses to angiotensin II (Doppler scanner and vein rings) were analyzed by two-way ANOVA with the factors genotype and drug dose and the Tukey *post hoc* test. Serum cTnI of patients was analyzed by two-way (genotype × time) ANOVA for repeated measures with the Tukey *post hoc* test for multiple comparisons. In addition, analysis of covariance including ejection fraction as a covariate was performed. The peak serum cTnI was compared by ANOVA. Investigators of skin microcirculation, vascular reactivity, myocardial damage, and Gq expression were blind as to the TT(-695/-694)GC genotypes. Differences were regarded statistically significant with an *a priori* alpha error $P < 0.05$.

Results

GC/GC Genotype Increased Cardiac Gq Expression

We measured Gq protein expression by Western Blot analysis using membrane preparations from human right atrial specimens (fig. 1, upper panel). Densitometric quantification of Gq protein expression in human right atrial specimens (n = 55) yielded a highly significant fold change of 1.27 for GC/GC *versus* TT/TT genotype carriers (fig. 1; $P = 0.006$).

Skin Perfusion

To investigate whether increased Gq expression in GC/GC genotype carriers translates into enhanced vasoconstriction

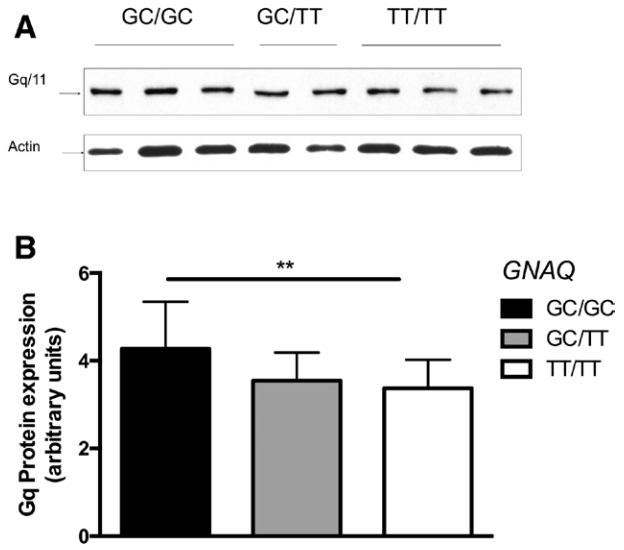

Fig. 1. Genotype-dependent Gq expression. (*A*) Lysate from cell membranes of right atrial specimens from patients with different *GNAQ* genotypes. Displayed is one representative blot probed with a Gq/11 antibody and, after stripping the blot, with an actin antibody as a control. (*B*) Relative quantification of Gq/11 expression by densitometry (mean ± SD) from experiments in right atrial specimens (n = 55: GC/GC, n = 24; GC/TT, n = 20; TT/TT, n = 11). $^{**}P < 0.01$ ANOVA.

after Gq activation *via* angiotensin receptor stimulation, skin perfusion changes were analyzed after intradermal injection of angiotensin II (10^{-16} and 10^{-14} M) in 18 healthy individuals (GC/GC, n = 5; GC/TT, n = 7; TT/TT, n = 6). Although baseline skin perfusion before injections was similar in different genotypes, angiotensin II evoked vasoconstriction with both angiotensin II concentrations only in GC/GC genotypes. In contrast, GC/TT genotypes showed a shifted dose–response curve with detectable vasoconstriction only after 10^{-14} M angiotensin II, and perfusion was almost unchanged in TT/TT-homozygous individuals ($P = 0.0002$ for comparison of genotypes, $P = 0.0003$ for GC/GC *vs.* TT/TT, and $P = 0.007$ for GC/TT *vs.* TT/TT; fig. 2A).

Angiotensin II–mediated Vasoconstriction in Isolated Human Saphenous Vein Rings

Angiotensin II–induced vasoconstriction was analyzed in vein rings obtained from patients undergoing coronary artery bypass grafting exposed to increasing angiotensin II in an organ bath. Angiotensin II resulted in an almost doubled maximum contraction in GC/GC homozygous compared with TT/TT carriers (62.9 ± 25.9% *vs.* 35.4 ± 21.7% of maximum KCl-evoked contraction, respectively, $P = 0.022$ for comparison of genotypes; fig. 2B).

Myocardial Injury

Baseline characteristics of the patients are presented in table 1. Genotype distribution (GC allele frequency 0.54) was comparable with that of healthy blood donors,[12] arguing against an association of *GNAQ* genotypes with increased

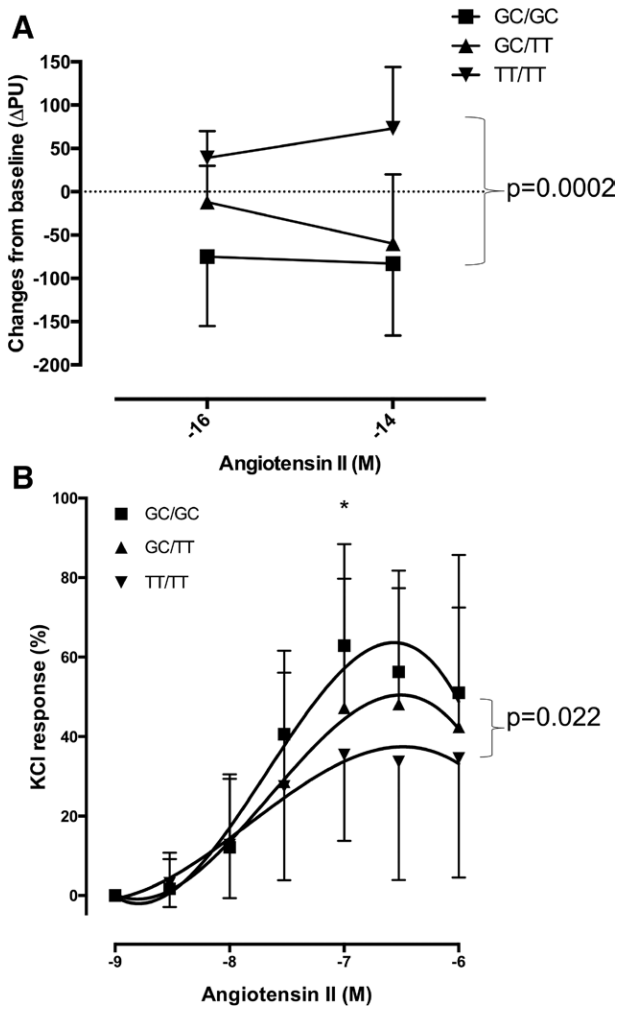

Fig. 2. Vascular response after intradermal angiotensin II injection. (*A*) Mean changes (±SD) in skin perfusion (expressed as changes from baseline of arbitrary perfusion units [ΔPU]) in response to angiotensin II, as stratified by *GNAQ* genotypes (GC/GC, n = 5; GC/TT, n = 7; TT/TT, n = 6). *P* value represents comparison of genotypes from two-way ANOVA (Tukey multiple comparison test yielded significant results for GC/GC *vs.* TT/TT and GC/TT *vs.* TT/TT). (*B*) Dose–response curves of contraction of saphenous vein rings in response to increasing angiotensin II concentrations (1 nM to 1 μM) according to *GNAQ* genotypes (GC/GC, n = 12; GC/TT, n = 28; TT/TT, n = 10). Curves were drawn with the use of a nonlinear fit-model (±SD) and a polynomial third-order equation. *P* value represents comparison of genotypes from two-way ANOVA ($^{*}P = 0.01$ for *post hoc* comparison for GC/GC *vs.* TT/TT).

susceptibility for coronary artery disease. Genotypes did not differ with regard to their demographics, risk factors, comorbidities, and medications. However, preoperative cardiac ejection fraction was significantly greater in patients with the GC/GC genotype (table 1). Intraoperative data such as bypass time, aortic cross-clamp time, and number of bypass grafts were all similar between different genotype carriers.

All patients presented postoperative increases of cTnI. Although preoperative cTnI did not differ between

Table 1. Perioperative Patient Characteristics of Patients Undergoing Coronary Artery Bypass Grafting

GNAQ Genotype	GC/GC	GC/TT	TT/TT	*P* Value
No. of patients	64	119	44	
Age, yr	68 ± 9	67 ± 9	68 ± 8.8	0.748
Body weight, kg	85 ± 16	83 ± 13	83 ± 17	0.395
Smoking				
Current	7 (11)	16 (13)	7 (16)	
Former	36 (56)	61 (51)	21 (48)	0.488
Preoperative creatinine serum concentration, mg/dl	1.2 ± 0.2	1.3 ± 0.5	1.3 ± 0.4	0.189
Systolic blood pressure, mmHg	135 ± 17	135 ± 21	134 ± 20	0.305
Diastolic blood pressure, mmHg	73 ± 11	75 ± 12	73 ± 12	0.909
Cardiac ejection fraction, %	55 ± 13	54 ± 14	48 ± 15	0.037
NYHA classification				
I-II	33 (52)	80 (67)	24 (55)	—
III	28 (44)	32 (27)	20 (46)	—
IV	3 (5)	7 (6)	0 (0)	0.385
Peripheral arterial disease	8 (13)	20 (17)	6 (14)	0.840
Left main coronary artery stenosis >50%	13 (20)	35 (30)	10 (23)	0.640
Preoperative cTnI >0.1 μg/l	4 (7)	12 (10)	3 (7)	0.826
No. grafts	3 ± 1	3 ± 1	3 ± 1	0.146
Internal mammary artery graft	63 (98)	106 (89)	42 (96)	0.362
Mitral valve insufficiency (moderate or severe)	6 (9)	10 (8)	4 (9)	0.935
Cardiopulmonary bypass time, min	126 ± 43	126 ± 42	127 ± 48	0.878
Aortic cross-clamp time, min	85 ± 26	83 ± 31	85 ± 33	0.937
Medication				
ASA	49 (79)	94 (82)	39 (89)	0.217
Clopidogrel	12 (19)	25 (22)	7 (16)	0.726
β-Blocker	52 (81)	99 (83)	34 (77)	0.672
Statins	38 (61)	83 (72)	29 (66)	0.510
ACEI/ARB	51 (80)	91 (76)	33 (75)	0.552
Diuretics	25 (38)	59 (50)	23 (52)	0.135
Calcium antagonists	12 (19)	27 (23)	11 (25)	0.426

Data are presented as means ± SD or no. (%).

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; ASA = acetylsalicylic acid; cTnI = cardiac troponin I; NYHA = New York Heart Association.

genotypes, postoperative cTnI was greatest in patients with the GC/GC genotype, followed by those with GC/TT and TT/TT genotypes, suggesting a gene–dose effect ($P = 0.034$ for comparison of genotypes; fig. 3). *Post hoc* analysis revealed a mean difference over time of 3.2 ng/ml between GC/GC and TT/TT genotypes ($P = 0.032$). Analysis of covariance including ejection fraction as a covariate did not change the results (mean difference over time between GC/GC and TT/TT genotype: 3.1 ng/ml; $P = 0.011$), thus demonstrating an independent association of the *GNAQ* polymorphism with cTnI. Peak cTnI after coronary artery bypass grafting almost doubled in GC/GC genotypes (11.5 ± 13.8 ng/ml) compared with heterozygous GC/TT (9.2 ± 9.2 ng/ml) and homozygous TT/TT genotypes (6.6 ± 4.8 ng/ml; $P = 0.015$).

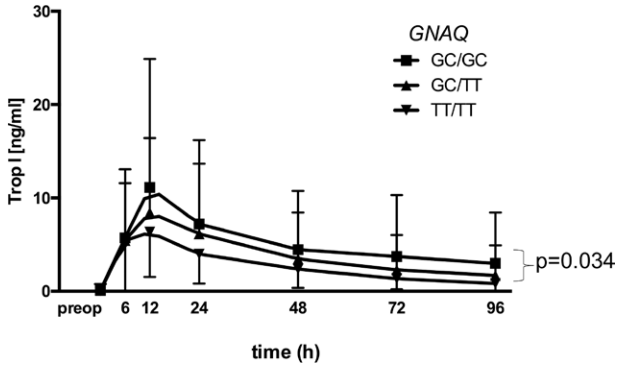

Fig. 3. Postoperative serum troponin I concentration (TropI) more than 96 h (mean ± SD) after coronary artery bypass grafting according to genotypes GC/GC (n = 64), GC/TT (n = 119), and TT/TT (n = 44). Peak cTnI was greater in GC/GC carriers compared with GC/TT and TT/TT genotypes ($P = 0.015$).

Discussion

Here we show that the substitution of TT for GC at positions -695/-694 of the dinucleotide polymorphism of the *GNAQ* gene promoter is associated with increased myocardial Gq protein expression. Moreover, we provide functional data showing that this polymorphism is functionally active: GC/GC genotype carriers show enhanced vasoconstrictor responses to the Gq activator angiotensin II in two different systems, skin capillary perfusion in volunteers and isolated

human saphenous veins *in vitro*. Finally, our data identify this dinucleotide polymorphism as a genetic risk factor for myocardial damage after coronary artery bypass grafting. These data, therefore, strongly support the clinical relevance of this *GNAQ* polymorphism.

Perioperative myocardial injury is attributed to transient myocardial ischemia–reperfusion and surgical injury and the acute inflammatory response associated with cardiopulmonary bypass.[18] Moreover, angiotensin II serum concentrations are increased during and after cardiopulmonary bypass and have been suggested to be involved in postoperative hypertension, potentially resulting in myocardial ischemia after surgery.[19,20]

The renin–angiotensin–aldosterone system is responsible for peripheral as well as central effects of vasoconstriction, and these effects are transmitted *via* angiotensin receptors.[7] In humans, attenuation of angiotensin II–mediated Gq signaling by angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers is a cornerstone of heart failure therapy.[21] It seems that chronic activation of the heterotrimeric G proteins Gq and G11 and their downstream signaling pathways is necessary and sufficient for myocardial hypertrophy. We and others have proposed that genetic variants may be associated with altered perioperative myocardial injury after coronary artery bypass grafting[22–25] but no data yet exist for the cardiac hypertrophy-related Gq pathway.

We previously characterized the *GNAQ* promoter and identified a dinucleotide TT(-695/-694)GC promoter polymorphism where both nucleotides always are exchanged simultaneously.[12] We also have shown that the GC allele displays increased binding to the transcription factor Sp-1 and is associated with enhanced promoter activity, increased Gq transcription, increased Gq-mediated intracellular signal transduction, and an increased prevalence of left ventricular hypertrophy.[12,26] This provided the first evidence that effects observed in transgenic mice may translate to the situation in human hearts.

However, because mRNA concentrations do not necessarily evoke corresponding changes of protein concentration, we extended our analyses and measured myocardial Gq protein along with functionally relevant phenotypes. Our current findings demonstrate that Gq protein expression is greatest in GC/GC genotype carriers, and this increased Gq expression translated into a measurable phenotype impacting on or reflecting perioperative myocardial injury. Measuring genotype-dependent differences of angiotensin II–induced vasoconstriction in different systems we could show enhanced vasoconstriction in GC/GC genotypes compared with GC/TTs or TT/TTs, suggesting a gene–dose effect.

Various signaling events are important both for the development and decompensation of left ventricular hypertrophy, and these involve cardiac paracrine and/or autocrine mediators like endothelin-1, norepinephrine, and/or angiotensin II, all of which act on cognate G protein-coupled receptors expressed in the myocardium.[27] Studies in transgenic mice show that the cardiomyocyte-specific overexpression of some of these G protein-coupled receptors, such as α_1-adrenergic and angiotensin type-1 receptors, or activated mutants of their coupled G-protein α -subunit Gq result in myocardial hypertrophy.[5]

Clinically, myocardial hypertrophy becomes evident and potentially has prognostic relevance especially in patients with coronary artery disease, implying reductions in coronary flow reserve in these patients.[28] Moreover, there is evidence that increased coronary microvascular tone, such as by α-adrenergic vasoconstriction, occurs more often in hearts with pathologic left ventricular hypertrophy, thereby reducing coronary blood flow with the risk of myocardial ischemia.[29]

Because global ischemia in hypertrophied hearts evokes increased troponin I concentrations,[10,11] we also investigated whether genotype-related differences in Gq expression are associated with altered perioperative myocardial damage after coronary artery bypass grafting surgery. Baseline characteristics of *GNAQ* genotypes showed greater ejection fraction in GC-allele carriers whereas factors, in particular with regard to clinical risk factors for perioperative myocardial damage, did not differ between genotypes across the study cohort. However, although the percentage of patients with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers was not different between genotypes and baseline arterial blood pressure also showed no difference, GC-homozygous patients may have received increased doses of those drugs to lower arterial blood pressure. Although, unfortunately, data collection about exact previous drug dosages was beyond the scope of our study, future investigations also should take into account this information.

Perioperative myocardial damage, as assessed by postoperative cTnI, was greatest in GC/GC-homozygous patients followed by GC/TT and TT/TT genotypes, again consistent with a gene–dose effect. One possible explanation for our observation could be that increased Gq expression in GC/GC genotype carriers results in left ventricular hypertrophy and decreased coronary reserve, rendering these individuals more susceptible to the detrimental effects of cardiac surgery. This hypothesis is supported by experiments in rats, where recovery of contractile function is depressed and lactate dehydrogenase or creatinine kinase activity was increased in hypertrophied hearts subjected to global ischemia, also suggestive of greater susceptibility to ischemia–reperfusion injury.[30–32]

Interestingly, a cross-talk between Gq and Gs signaling pathways has been proposed,[33] and increased Gq expression has been shown to decrease cAMP production through Gs protein ubiquitination and its proteasomal degradation.[34,35] Although cAMP represents a critical regulator for left ventricular contractile function, facilitated Gs degradation and depressed cAMP production by increased Gq expression in GC-allele carriers may represent a novel mechanism for Gq-induced cardiac dysfunction after coronary artery bypass grafting.

Our results may have clinical implications. Although we have shown previously that remote ischemic preconditioning protects the heart from ischemic damage,[36] two large multicenter trials failed to show a cardioprotective effect of remote ischemic preconditioning.[37,38] Given that a certain *GNAQ* genotype is associated with altered postoperative cTnI, one could argue that the remote ischemic preconditioning effect may be detectable in selected genotype carriers only. Although we can only speculate on this topic, future studies are necessary to investigate a potential interaction of remote ischemic preconditioning with the *GNAQ* polymorphism.

Another potential clinical implication is the therapy with a vasodilator, *e.g.,* therapy with vascular endothelial growth factor, a treatment option for heart failure. Here, vascular endothelial growth factor gene therapy in patients with coronary artery disease hitherto has not demonstrated a clinical benefit.[39] However, it might be speculated from our data that only certain individuals, such as GC-homozygous patients for the TT(-695/-694)GC polymorphism, may benefit from vasodilator therapy because those individuals have a high level of coronary vasoconstriction. Those individuals also may benefit from other postoperative vasodilator therapies, such as with endothelin-receptor blockers. However, these questions were beyond the scope of the present study.

Some limitations must be addressed. First, we speculate that increased myocardial damage observed in GC/GC carriers during coronary artery bypass grafting surgery may be due to left ventricular hypertrophy. Although we did not measure hypertrophy-related echocardiographic parameters, this was not tested directly. However, we already had shown in our previous study that the polymorphism is indeed associated with left ventricular hypertrophy[12] and therefore assume the same mechanism for the current study. Second, we were not able to perform a reasonable *a priori* power analysis because this is the first analysis of *GNAQ* genotypes regarding postoperative myocardial damage as well as vasoconstriction response after angiotensin II stimulation. Therefore, our results should be regarded as a pilot study, and future studies may take these results into account to calculate an appropriate *a priori* power analysis.

In conclusion, our results demonstrate that the functionally relevant *GNAQ* TT(-695/-694)GC promoter polymorphism evokes increased myocardial Gq expression, enhanced vasoconstrictor responses in skin and isolated veins after angiotensin II stimulation, and increased perioperative myocardial damage after coronary artery bypass grafting. Thus, our data shed new light on the role of genetically evoked altered Gq expression in ischemic heart disease as well as for human vasomotor responses and may help to identify patients at greater risk for myocardial injury after coronary artery bypass grafting.

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Competing Interests

The authors declare no competing interests.

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