Published in final edited form as:

Pharmacogenomics. 2010 September ; 11(9): 1209–1221. doi:10.2217/pgs.10.88.

β -blocker therapy and heart rate control during exercise testing in the general population: role of a common G-protein β -3 subunit variant

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Abstract

Aim—Impaired heart rate (HR) response to exercise is associated with increased cardiovascular morbidity and mortality. We analyzed whether common variants (rs5443/C825T and rs5442/G814A) in the G-protein β 3 subunit (*GNB3*) gene modulate interindividual variation in β -blocker responses with respect to HR.

Materials & methods—Among 1614 subjects (347 current β -blocker users) of a population-based study, HR during symptom-limited exercise testing was analyzed by multilevel linear regression models adjusted for potential confounders.

Results—In β -blocker users, but not in nonusers, HR was attenuated in rs5443 T allele carriers (TC/TT vs CC) with lower adjusted HR over the entire exercise period from rest to peak workload (3.5 bpm; 95% CI: 1.1–5.8; $p < 0.01$), and during recovery (4.2 bpm; 95% CI: 0.6–7.8; $p = 0.02$). The genotype-related HR reducing effect at peak exercise varied by up to 7.5 bpm (CC vs TT),

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Financial & competing interests disclosure: The work is part of the Community Medicine Research net (CMR) of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grant no. ZZ9603), the Ministry of Cultural Affairs, and the Social Ministry of the Federal State of Mecklenburg-West Pomerania. The CMR encompasses several research projects, which share data from the population-based Study of Health in Pomerania (SHIP;

<http://www.medizin.unigreifswald.de/cm>). This work is part supported by the research project Greifswald Approach to Individualized Medicine (GANI_MED). The GANI_MED consortium is funded by the Federal Ministry of Education and Research and the Ministry of Cultural Affairs of the Federal State of Mecklenburg, West Pomerania (03IS2061A). This study was further supported by the future fund of the state government of Mecklenburg-Vorpommern (UG 07 034) and by NIDCR/NIH grant DE0160057. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research: The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

more than a third (35.9%) of the total β -blocker effect (20.9 bpm). By contrast, rs5442 had no impact on any HR-related parameter.

Conclusion—In this population-based sample, a common *GNB3* polymorphism (C825T) was significantly related with response to β -blocker therapy with respect to HR during exercise and HR recovery, respectively. Further prospective studies are needed to confirm these associations and to examine their potential clinical relevance.

Keywords

β -blocker; epidemiology; exercise testing; genetic susceptibility; *GNB3*; heart rate; polymorphism

β -blockers are among the most frequently prescribed cardiovascular drugs and are a pillar in the treatment of chronic heart failure and post-myocardial infarction. In accordance with current guidelines more than 90% of all patients with myocardial infarction in the USA are discharged from hospital with β -blockers [1]. While some trials suggest administering the highest tolerated β -blocker dose in these patients [2], former and current concepts still highlight heart rate (HR) reduction as main predictor for the beneficial effects of chronic β -blocker therapy irrespective of dosage, and suggest individualized dose-titration regimens [3–5].

The use of β -blockers as first-line therapy for uncomplicated hypertension, however, is discussed controversially [6,7], since an association with a greater risk of cardiovascular events has been reported [6]. Furthermore, hypotension and bradycardia – among other adverse effects – contribute to the declining adherences to β -blocker therapy and to dosage reductions below recommendations [5,8]. In this context, perioperative β -blockade in noncardiac surgery has recently been challenged by the Postischemic Evaluation Study (POISE) trial, where the advantage of lower incidence rates of nonfatal myocardial infarctions was diminished by an increase of adverse events during follow-up (higher incidences of stroke, hypotension, bradycardia and increased all-cause mortality) [9].

Heart rate control, particularly under exercise conditions, is a highly complex physiological process. Genetic influences and environmental factors affect interindividual variability [10]. Identification of genetic factors contributing to resting HR, HR regulation during exercise and HR recovery in the presence of β -blocker therapy may have an impact on dose finding in general practice, which is commonly based on an empirical titration process, and could facilitate the differentiation of subjects that will benefit from individuals at risk for adverse events.

In this context, a common variant (rs5443; C825T) in the G-protein $\beta 3$ subunit (*GNB3*) gene is a promising candidate gene for modulation of HR control under β -blocker therapy. Heterotrimeric G-proteins are key signal transducers for a large family of membrane receptors, such as adrenoceptors, the muscarinic acetylcholine receptors, and those for angiotensin II, endothelin, bradykinin and vasopressin [11]. *GNB3* variants have been associated with different cardiovascular and metabolic disorders and have been shown to affect pharmacologic response to different drugs [12–15]. Several reports have related *GNB3* C825T to varying α - and β -adrenergic responses [14,16–18], antihypertensive effects of β -blockers [19] and altered autonomous functions [18,20].

Using a large population-based sample of Caucasian adults, the purpose of this study was to investigate whether common *GNB3* variants modulate HR control in the presence of β -blocker therapy during exercise testing.

Materials & methods

Participants

The design of the population-based Study of Health in Pomerania (SHIP) has been published previously [21]. Briefly, SHIP is a population-based study representative of the population in the northeast area of Germany. A total of 4310 baseline subjects were examined between 1997 and 2001. The study was approved by the ethics committee of the University of Greifswald, and all participants gave informed written consent. The first follow-up examination (SHIP-1) was conducted 5 years (median: 5.04 ± 0.62 years) after baseline and comprised of 3300 subjects (83.6% which are still living subjects).

During March 2003 and October 2006, 1707 subjects volunteered once for exercise testing. A total of 41 subjects (2.4%) were excluded because of missing *GNB3* genotypes owing to lack of DNA, objections to DNA analyses or genotyping failures. Furthermore, we excluded individuals with pacemakers ($n = 7$), atrial fibrillation ($n = 14$), uncertainty regarding β -blocker medication ($n = 1$), and moderately or severely limited left ventricular function ($n = 30$) defined by an echocardiographic fractional shortening less than 22% in women and less than 20% in men [22]. There were no heart transplanted patients among the final study sample that included data of 1614 participants (94.6% of those undergoing exercise testing).

Clinical data

Sociodemographic characteristics and medical histories were assessed by computer-aided personal interviews. Hypertension was defined as a resting systolic blood pressure (BP) of 140 mmHg or more, a diastolic BP of 90 mmHg or more or use of antihypertensive medication. As for smoking status, participants were classified as never smoker, exsmoker or current smoker. They were considered physically active if they participated in physical training for at least one hour a week [23]. Height and weight were measured for the calculation of BMI (weight [kg]/height² [m²]). Medication, including doses was recorded by a computer-aided method using the Anatomical Therapeutic Chemical Classification System[®] (ATC) [101]. Daily β -blocker equal doses were calculated based on the daily-defined-dose concept of the ATC using the most common β -blocker agent among our sample, bisoprolol, as reference [101]. All affected subjects were on the current β -blocker doses for at least 7 days, defined as chronic β -blocker therapy in our study.

Genotyping

DNA was extracted from whole blood, and the *GNB3* C825T (rs5443) polymorphism was analyzed as recently described [24]. The rs5442 polymorphism was genotyped by the SNPlex[®] assay [25]. The genotyping laboratories were unaware of any clinical data of the participants.

Genetic variance at the *GNB3* locus

The *GNB3* gene is short, encompassing approximately 7210 nucleotides. It is represented by one genetic block, and the entire genetic variance is limited. Most association studies on *GNB3* focused on the functional C825T polymorphism (rs5443) [12,26]. In Caucasians, the C825T polymorphism is in almost complete linkage disequilibrium with five other polymorphisms (rs589231113, rs11064426, rs2301339, rs13306405 and rs5446) (Supplementary Figure 1; www.futuremedicine.com/doi/suppl/10.2217/pgs.10.88) [26]. Together, they constitute two principal haplotypes commonly termed C and T, following the nomenclature of the C825T key polymorphisms [26]. rs5442 (G814A), located adjacent to rs5443 is another common *GNB3* variant occurring at a minor allele frequency of 5–10% in Caucasians (6.16% in our study population). The A allele of rs5442 occurs virtually in exclusive combination with the rs5443 C allele, while the frequent rs5442 G allele manifests

with both rs5443 alleles. This allows the subdivision into T (rs5442G–rs5443T), C1 (rs5442G–rs5443C), and C2 (rs5442A–rs5443G) haplotypes (Supplementary Figure 1). The functional significance of the rs5442 variant has not been addressed so far, although it contains an amino acid exchange in a highly conserved motif present in the G β 1–G β 4 family. However, when we subdivided into C1 and C2 haplotypes, findings of this study did not vary. Thus, we present solely the results for the rs5443 C825T polymorphism in this article. The Supplementary material lists further detailed data (see www.futuremedicine.com/doi/suppl/10.2217/pgs.10.88).

Exercise testing

Maximal symptom-limited exercise testing was performed using electromagnetically braked cycle (Ergoselect 100, Ergoline, Bitz, Germany) according to the Jones protocol (stepwise increases in workload of 16 Watt/min, starting with unloaded cycling plus the ergometer-related permanent load) [27]. Electrocardiographic monitoring was performed continuously. HR and BP were obtained at rest, at the end of each stage of exercise and during the first 3 min of recovery.

Chronotropic response

The primary aim of the study was to investigate HR during exercise. For that purpose, each subject's exercise test was divided into five consequent periods of equal duration (rest, 20, 40, 60, 80 and 100% of maximal workload) [28]. Maximal workload was defined as the workload during the last completely finished stage of exercise and age-predicted maximal workload was calculated by the routine algorithm of the ergometer based on standard equations [29]. Peak HR was defined as the maximal HR achieved. Age-predicted maximum HR was estimated by the equation $220 - \text{age}$ [30], which is frequently used in exercise studies [28,31,32]. This formula is also the only one that has been applied in context of β -blocker therapy in a large epidemiological study [31]. Since its validity in different populations has been controversially discussed [33], we additionally applied the equation $205.8 - 0.685 \times \text{age}$ [34], which has been proposed as the most accurate general formula [33]. The percentage of maximum age-predicted HR was calculated as the ratio between peak HR and predicted maximum HR for both equations separately. HR reserve was calculated as the difference between maximal age-predicted HR and resting HR. The chronotropic index was calculated as the percentage of HR reserve used at peak exercise ($\text{peak HR} - \text{resting HR} / \text{HR reserve}$) [31,32].

Statistical analyses

Descriptive comparisons between genotypes were made using χ^2 -test (nominal data) and one-way analysis of variance (ANOVA) (continuous data) with Bonferroni's *post hoc* test. Values are given as means $\pm$ standard deviation (SD) or absolute numbers (percentages). Two-level multilevel linear regression models, using the xtreg command of the statistical software package STATA, with timepoints at level one and individuals at level two, were fitted to estimate the contribution of the genotypes on HR response during exercise (rest, 20, 40, 60, 80 and 100% of maximal workload), and on HR recovery (maximal workload, 1, 2 and 3 min of recovery). This approach is advantageous as it accounts for the correlated data structure across timepoints. Analyses were stratified by β -blocker use. A first model controlled for age and gender. Subsequently, the influence of a large set of confounders was tested: physical activity, BMI, hypertension, systolic and diastolic BP, smoking status, crucial medication (e.g., calcium-channel blockers, angiotensin-converting enzyme [ACE] inhibitors, angiotensin receptor blockers, digitalis and amiodarone), and a summative index of comorbidities (including stroke, diabetes mellitus, chronic obstructive pulmonary disease [COPD], myocardial infarction, asthma, peripheral vascular disease, liver diseases, osteoporosis, gout, rheumatic disorders, thyroid disorders and depression). For β -blocker

users, models were additionally controlled for β -blocker doses. Control variables other than gender and age were removed in a backward stepwise strategy when the p-value for single parameters was more than 0.20. The following variables were retained in the model during exercise: age, sex, systolic and diastolic BP, hypertension, smoking status, use of ACE inhibitors and digitalis, and β -blocker doses among β -blocker users. In addition to these variables amiodarone use was retained in the model during recovery.

Because estimates for *GNB3* genotype differed very little between the age- and gender-controlled model and the full covariate model, only the latter is presented. Furthermore, adding comorbidities separately into the regression models instead of the global comorbidity index did not affect our results. Wald tests were applied to test multiparameter effects concerning the dummy-coded *GNB3* variable (CC genotype as reference category) and its interaction with time. An autoregressive covariance structure was applied to model dependencies between measurements.

To assess the sensitivity of our results to systematic nonresponse in exercise testing and recovery we additionally applied statistical weights. The weights accounted for systematic differences in participants and nonparticipants of exercise testing regarding the variables presented in Table 1.

Estimated HR values are given as adjusted means and 95% CI. A p-value of less than 0.05 was considered statistically significant. Analyses were performed with STATA 10 (StataCorp LP, College Station, TX, USA) and SPSS software version 15.0.1 (SPSS Inc., IL, USA).

Results

Baseline characteristics

Table 1 presents baseline characteristics of subjects without (nonparticipants) and with exercise testing (study population). Nonparticipants were older, had a higher frequency of comorbidities (diabetes mellitus, hypertension, myocardial infarction, asthma bronchiale and COPD) and a higher frequency of taking medication (including β -blockers). Moreover, they were more frequently current smokers, more often physically inactive, had a higher BMI and a lower resting HR (Table 1).

Among the study population of 1614 participants (828 women) with exercise data, 347 (180 women) had a current β -blocker medication, and 1267 had not. There were no statistically significant differences between subjects with and without β -blocker therapy regarding the genotype frequencies of *GNB3* rs5443 (Table 1) and *GNB3* rs5542 (AA: 0.0 and 0.7%; AG: 11.2 and 11.0%; GG: 87.7 and 80.7%; $p = 0.788$), respectively. Genotype distributions were in accordance with a Hardy–Weinberg equilibrium ($p = 0.84$ and $p = 0.87$, respectively) as well as in line with previous data for German populations [26,35].

The distribution of β -blocker agents and daily equal doses did not differ between the *GNB3* C825T genotypes (Table 2). Stratified analyses with respect to β -blocker usage did not reveal any significant associations between the *GNB3* C825T genotype and baseline characteristics with the exception of a slightly lower systolic BP at rest in TT allele carriers among β -blocker users (Table 3). As already indicated, *GNB3* G814A genotypes were not associated with any of the baseline characteristics in both groups (Supplementary Material).

Exercise data

Both exercise duration and maximal workload were lower in subjects with β -blockers compared with those without β -blockers (7.9 ± 2.7 min versus 9.4 ± 3.1 min and $134.0 \pm$

43.2 Watt (W) versus 158.9 ± 50.2 W; $p < 0.001$, respectively). These differences were still present after adjustment for age and gender (8.8 min, 95% CI: 8.6–9.0 versus 9.2 min, 95% CI: 9.1–9.3; $p = 0.002$, and 148.1 W, 95% CI: 144.4–151.7 versus 155.0 W, 95% CI: 153.2–156.8; $p = 0.001$).

Stratified analyses with respect to the β -blocker status did not reveal any statistically significant differences between the *GNB3* genotypes for exercise duration, maximal power load, age predicted workload reached and age-predicted HR reached (Table 3).

Use of β -blockers, *GNB3* polymorphisms, HR response & chronotropic incompetence

Compared with participants without β -blockers, those with β -blockers had a lower HR at any timepoint of the exercise regardless of the *GNB3* genotype ($p < 0.001$). After adjustment for all selected confounders the maximum HR at peak exercise was 134.1 bpm (95% CI: 131.8–136.4) in β -blocker users, and 155.0 bpm (95% CI: 154.2–155.8) in non-users ($p < 0.001$). Thus, the maximum achieved β -blocker effect on HR reduction was 20.9 bpm.

Among subjects without β -blockers, *GNB3* genotypes were not associated with HR during any stage of exercise (Figure 1). By contrast, *GNB3* genotypes were consistently related to HR during exercise among subjects with β -blockers (Figure 1). Thus, subjects with TC and TT genotype exhibited a reduced HR compared with carriers of the CC genotype. The average HR over the entire exercise period from rest to peak workload was 2.9 bpm (95% CI: 0.4–5.4, $p = 0.02$) lower in subjects with the TC genotype, and 5.8 bpm (95% CI: 1.9–9.7, $p < 0.01$) lower in subjects with the TT genotype compared with the CC genotype. Testing heterozygote and homozygous T allele carriers together (recessive genetic model), revealed a 3.5 bpm (95% CI: 1.1–5.8, $p < 0.01$) lower average HR compared with noncarriers with the T allele.

Heart rate differences between *GNB3* genotypes were largest at peak exercise. The adjusted HR was 4.7 bpm (95% CI: 0.7–8.7, $p = 0.02$) lower in subjects with the TC genotype, and 7.5 bpm (95% CI: 1.2–13.9, $p = 0.02$) lower in subjects with the TT genotype in comparison to the CC genotype. This equaled approximately 35.9% of the maximum β -blocker effect observed (20.9 bpm) between the entire population of β -blocker users and the entire population of the nonusers of β -blocker therapy. Figure 2 illustrates the maximum effects of β -blocker therapy on HR reduction for all β -blocker users as well as stratified for the *GNB3* genotype in comparison to the maximum HR in subjects without β -blockers.

In addition, compared with subjects with the CC genotype (0.66, 95% CI: 0.63–0.70), the adjusted chronotropic index tended to be lower in subjects with the TC (0.62, 95% CI: 0.59–0.65) and the TT (0.59, 95% CI: 0.53–0.66) genotype. This difference was borderline statistically significant after controlling for the resting HR (TT/TC vs CC; $p = 0.05$).

Again, HR and chronotropic incompetence were not affected by the *GNB3* G814A polymorphism, neither if analyzed as single variant, or upon subdifferentiation of the *GNB3* C haplotype (Supplementary Material).

HR recovery

Similar to HR during exercise, there was an association between the *GNB3* genotype and HR during recovery among participants with β -blockers, but not among those without β -blockers (Figure 3). The average HR during recovery in subjects with the TC and the TT genotype was 4.2 bpm (95% CI: 0.3–8.2, $p = 0.03$), and 4.3 bpm (95% CI: 1.3–9.8, $p = 0.13$) lower compared with the CC genotype. Overall, T allele carriers (TT/TC) had a 4.2 bpm (95% CI: 0.6–7.8, $p = 0.02$) lower HR compared with noncarriers during recovery. These effects were largest at the beginning of the recovery phase, and were independent from peak

HR during exercise (Figure 3). Again, *GNB3* G814A genotype was not significantly associated with HR during recovery (Supplementary Material).

Discussion

Our study is one of the first that addresses issues of pharmacogenomics in a population-based survey. We present evidence that a common *GNB3* variant, rs5443 C825T, is associated with HR during rest and any stage of exercise testing in subjects with β -blocker therapy. In particular, the T allele was associated with lower HR during both exercise and recovery. HR differences related to *GNB3* were particularly pronounced at peak exercise. The genotype-related variance in HR reduction of more than 7.5 bpm between the TT and the CC genotype at peak exercise equaled to more than a third of the total β -blocker effect of 20.9 bpm that was observed in the entire population of β -blocker users compared with the entire group of nonusers.

The potential impact of *GNB3* on hemodynamic regulation has been corroborated with some evidence in previous studies [14,16–18,36–39]. Recently, a study in 79 young healthy subjects reported a greater HR response during a cold pressure test in homozygous carriers of the *GNB3* 835T allele [18]. Two other studies have suggested that *GNB3* variants may modulate β -blockers effects [17,19]. Schäfers *et al.* reported an association of the *GNB3* T allele with acute hemodynamic effects after administration of propranolol in 71 young healthy males, including lower total peripheral resistances and higher cardiac indices [17]. Another study demonstrated genotype-specific differences of a chronic β -blocker therapy on BP lowering with stronger effects in T allele carriers [19].

Interestingly, in our study HR during rest and exercise was not modulated by *GNB3* genotypes in β -blocker-naïve subjects, which is in line with a number of previous studies on resting HR [15,40]. By contrast, resting HR was lower in T allele carriers compared with homozygous CC carriers (57.4 ± 1.9 vs 61.1 ± 1.7 bpm) as seen in the study by Schäfers *et al.* [17]. Similar findings were reported for resting HR in the Hypertension and Ambulatory Recording Venetia (HARVEST) study among 461 untreated hypertensives aged 18–45 years (76.4 ± 0.7 bpm in CC genotypes vs 73.2 ± 1.6 bpm in TT genotypes) [41], although these differences did not reach statistical significance. In addition to our study, the Health, Risk factors, exercise Training and Genetics (HERITAGE) Family Study was the only one to address genotype-dependent HR response during exercise in normotensives without medication [42]. In this study, contrary to our results, the CC genotype was associated with a lower resting HR and a greater training-induced reduction in HR in 255 black subjects. However, there were no differences between the CC, TC and TT genotypes with respect to resting HR (64.0 ± 1.0 , 64.4 ± 1.0 and 63.6 ± 1.7 bpm; $p = 0.821$) or HR during a submaximal cycle ergometer test at 50 W (117.4 ± 1.8 , 116.4 ± 2.1 and 113.3 ± 2.4 bpm; $p = 0.156$) in 473 Caucasians. Thus, these findings are in line with those observed in our study for β -blocker-naïve subjects at submaximal exercise stages.

The potential molecular mechanisms underlying our findings remain to be established. Available evidence suggests that the *GNB3* 825T allele – a silent polymorphism with regard to the amino acid sequence – favors alternative splicing, ultimately resulting in two protein variants termed G β 3s and G β 3s225. The pronounced expression of these splice variants in context of the T allele is associated with increased signal transduction [12,14,16,26,38,43]. Because G proteins are involved in the signaling cascades of more than a thousand different receptors, complex phenotypes in association with the Gb3 rs5443 alleles have been described [15]. While for agonists straight forward hypotheses on the actions of the 825T allele may be constructed, chronic effects of an antagonist like chronic β -receptor blockers are difficult to predict. Long-term administration of β -blockers results in enhanced β -

adrenoceptor expression [44]. The ratio of β 1- and β 2-receptor changes and receptor-mediated sensitization processes are influenced by G proteins [11]. It may be speculated that in exercise testing with increasing levels of catecholamines and long-term administration of β -blockers, the degree of G-protein-mediated signaling is most likely to govern the levels of cellular and physiologic effects. Furthermore, long-term β -blockade affects not only the adrenergic system but also central brain and parasympathetic systems. Therefore, a role for effective signal transduction linked to the rs5443 polymorphism appears plausible.

Moreover, it has to be taken into account that classes of β -blockers differ with regard to the degree of β 1-selectivity, intrinsic sympathomimetic activity or parallel α 1-blocking effects. The vast majority of β -blocker agents used in our study were metoprolol and bisoprolol. Although the distribution of β -blockers between the *GNB3* genotypes was not statistically different, we cannot completely exclude that this might have influenced our results given the high variation in receptor affinity of different β -blocker generations. Analyses stratified for different β -blocker classes were not feasible owing to low statistical power. Accumulating evidence suggests that common β -blockers, including metoprolol, bisoprolol and carvedilol are better regarded as inverse agonists than pure antagonists [45]. In this scenario, ligand, receptor and G proteins interact to control their mutual affinities, a process potentially affected by *GNB3* variants. While such a mechanism awaits experimental verification for G β proteins, elegant studies have actually shown that genetic variants in β -receptors affect the inverse antagonistic behavior of β -blockers [46].

Our study aim was not to identify new polymorphisms that might modulate β -blocker response. But rather we followed a candidate-gene approach and focused on potential effects of a common G-protein variant on HR control of β -blockers. Of course, other gene polymorphisms, for example, genes of the β 1-receptor, β 2-receptor or CYP2D6 in the case of metoprolol, also play an important role [45]. These questions should be addressed by prospectively designed studies.

The potential clinical relevance of our findings can only be speculated. On the one hand, decreased resting HR has been associated with beneficial effects, including reduced all-cause and cardiovascular mortality in both the general population and in various cardiovascular diseases [4,28]. In the Metoprolol CR/XL Randomised Intervention Trial in congestive Heart Failure (MERIT-HF) trial, a reduction of total mortality in 1845 heart failure patients was independent from the maximum β -blocker dose reached, meaning that HR reduction was the main predictor of beneficial β -blocker therapy [5]. Conversely, the attenuated HR during exercise and the lower chronotropic index (i.e., chronotropic incompetence), as seen in T allele carriers of our study, might theoretically point toward an adverse prognosis [6,9,31,32]. Thus, chronotropic incompetence was associated with increased total and cardiovascular mortality in various studies in β -blocker-naïve subjects [28,31,32,47] as well as with increased all-cause mortality among 3736 patients taking metoprolol tartrate or atenolol in one study [31]. However, it is not feasible to conclude whether the effects observed in our study are advantageous or detrimental, and how it could impact future clinical practice.

Several potential limitations of our investigation merit comment. All subjects volunteered for exercise testing, implying a potential selection bias. Therefore, we additionally applied weights that accounted for systematic differences in participants and nonparticipants of exercise testing. These sensitivity analyses did not suggest a different association between the *GNB3* C825T genotype and HR during exercise and recovery. Moreover, it should be noted that no information on the indication for β -blocker therapy was available. With respect to the baseline characteristics, however, it may be assumed that the main indications were hypertension and previous myocardial infarctions (Table 1). Based on the study design we

are not able to provide any information on potential contraindications for β -blockers or discontinuation of a previous β -blocker therapy among subjects that were not on this medication during the current examination. Strengths of our study include the availability of extensive data on key risk factors, particularly on β -blocker dosages, comorbidities and on echocardiographically assessed left ventricular function as well as the consistency of the results over multiple statistical models. Despite uncontrollable confounding factors, which are necessarily present in a population-based scenario, associations between the *GNB3* C825T genotype and HR control of β -blockers were significant over various statistical models. Repeating our analyses with different combinations of confounders would not have altered our substantial conclusions.

Conclusion

We have demonstrated that a common polymorphism of the *GNB3* gene modulates HR control of β -blockers. These findings have to be confirmed and the potential clinical relevance has to be investigated in further studies. Identification and confirmation of such gene–drug interactions may potentially contribute to future pharmacogenetically based dosage regimens for β -blockers or other drugs to facilitate individual dose adjustment, thereby potentially reducing adverse effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The contribution to data collection made by field workers, technicians, interviewers and computer assistants is gratefully acknowledged.

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Figure 1. β -blocker use, *GNB3* C825T genotype and heart rate response during exercise

Full model adjusted for age, sex, systolic and diastolic blood pressure, hypertension, smoking status, use of angiotensin-converting enzyme inhibitors and digitalis, and β -blocker doses among β -blocker users.

p-values at specific time points of exercise (multilevel linear regression model).

†p < 0.05; ‡p < 0.05; §p < 0.05; ¶p < 0.01 (TC/TT vs CC).

Figure 2. Heart rate reduction at peak exercise in β -blocker users compared with nonusers. Heart rate reduction at peak exercise in β -blocker users compared with nonusers for all subjects (blue column) and stratified by *GNB3* genotype (green column for CC, light purple column for TC and purple column for TT). Values are means adjusted for age, sex, systolic and diastolic blood pressure, hypertension, smoking status, use of angiotensin-converting enzyme inhibitors and digitalis, and β -blocker doses among β -blocker users.
[†] $p < 0.05$; [‡] $p < 0.01$ (multilevel linear regression model).

Figure 3. β -blocker use, *GNB3* C825T genotype and heart rate recovery

Full model: adjusted for age, sex, systolic and diastolic blood pressure, hypertension, smoking status, use of angiotensin-converting enzyme inhibitors and digitalis, and β -blocker doses among β -blocker users. p-values at specific time points of exercise (multilevel linear regression model).

$^{\dagger}p < 0.05$; $^{\ddagger}p < 0.05$; $^{\S}p < 0.05$, $^{\P}p < 0.01$ (TC/TT vs CC).

Table 1

Baseline characteristics of subjects without and with exercise testing.

Characteristic	No exercise testing (n = 1415)	Exercise testing (study population)		
		All subjects (n = 1614)	No β -blocker (n = 1267)	β -blocker (n = 347)
Age, mean (SD)	56.1 (16.6)	51.9 (13.5)	49.6 (13.2)	60.1 (11.2)
Women, n (%)	663 (46.9)	828 (51.3)	648 (51.1)	180 (51.9)
Diabetes mellitus, n (%)	190 (13.4)	116 (7.2)	50 (3.9)	54 (15.6)
Hypertension, n (%)	809 (57.2)	759 (47.1)	438 (34.6)	290 (83.9)
Myocardial infarction, n (%)	70 (4.9)	42 (2.6)	11 (0.9)	31 (8.9)
Stroke, n (%)	49 (3.5)	24 (1.5)	12 (0.9)	12 (3.5)
Asthma bronchiale, n (%)	55 (3.9)	37 (2.3)	30 (2.4)	7 (2.0)
COPD, n (%)	93 (6.6)	67 (4.1)	49 (3.9)	18 (5.2)
Smoking status		0.022		<0.001
– Never smoker, n (%)	558 (39.7)	703 (43.6)	536 (42.3)	168 (48.6)
– Exsmoker, n (%)	447 (31.8)	513 (31.8)	377 (29.8)	136 (39.3)
– Current smoker, n (%)	399 (28.4)	396 (24.6)	354 (27.9)	42 (12.1)
Physically active, n (%)	157 (11.1)	274 (17.0)	589 (46.5)	139 (40.2)
BMI (kg/m ²), mean (SD)	28.1 (5.1)	27.7 (4.7)	28.0 (4.3)	28.1 (4.8)
Blood pressure (mmHg)				
– Systolic, mean (SD)	135 (22)	130 (18)	129 (17)	137 (19)
– Diastolic, mean (SD)	81 (11)	82 (10)	81 (11)	82 (11)
Resting heart rate, mean (SD)	69.7 (11.0)	78.4 (13.3)	69.6 (9.8)	62.7 (9.6)
Fractional shortening (%), mean (SD)	37.5 (7.4)	37.7 (7.6)	37.6 (7.6)	38.4 (8.0)
GNB3 C825T genotype		0.605		0.873
– CC	674 (47.6)	757 (46.9)	600 (47.3)	157 (45.3)
– TC	593 (41.9)	694 (43.0)	543 (42.9)	151 (43.5)
– TT	148 (10.5)	163 (10.1)	124 (9.8)	39 (11.2)
Medication				
β -blocker, n (%)	390 (27.6)	347 (21.5)	<0.001	0 (0.0)
				347 (100.0)

Characteristic	No exercise testing (n = 1415)	Exercise testing (study population)		
		All subjects (n = 1614)	p-value [†] (n = 1267)	β-blocker (n = 347)
Calcium-channel blocker			<0.001	<0.001
– Diltiazem or verapamil, n (%)	35 (2.5)	11 (0.7)	10 (0.8)	1 (0.3)
– Dihydropyridine, n (%)	130 (9.2)	95 (5.9)	50 (3.9)	45 (13.0)
Digitalis, n (%)	52 (3.7)	17 (1.1)	7 (0.6)	10 (2.9)
Amiodarone, n (%)	5 (0.4)	1 (0.1)	0 (0.0)	1 (0.3)
ACE inhibitor, n (%)	314 (22.2)	252 (15.6)	119 (9.4)	133 (38.3)
Angiotensin receptor blocker, n (%)	91 (6.4)	88 (5.59)	40 (3.2)	49 (14.1)
Any antiobstructive drug	86 (6.1)	69 (4.3)	52 (4.1)	17 (4.9)
– β-adrenoceptor agonist, n (%)	31 (2.2)	29 (1.8)	25 (2.0)	4 (1.2)
– β2-adrenoceptor agonist, n (%)	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
– α-adrenoceptor antagonist, n (%)	21 (1.5)	4 (0.2)	0 (0.0)	4 (1.2)

Given values are unadjusted. Only subjects without exclusion criteria for the analyses are considered.

[†] Subjects without versus with exercise data.

[‡] No β-blocker versus β-blocker among subjects with exercise data (χ^2 -test or one-way ANOVA test with Bonferroni's post-hoc test, as appropriate).

ACE: Angiotensin-converting enzyme; ANOVA: Analysis of variance; COPD: Chronic obstructive pulmonary disease; SD: Standard deviation.

Table 2

β-blocker agents, daily doses and *GNB3* C825T genotype.

β-blocker agent	CC (n = 157)	TC (n = 151)	TT (n = 39)
Atenolol, n (%)	4 (2.5)	8 (5.3)	3 (7.7)
Metoprolol, n (%)	3 (1.9)	1 (0.7)	1 (2.6)
Metoprolol succinate or tartrate, n (%)	46 (29.3)	40 (26.5)	8 (20.5)
Bisoprolol, n (%)	71 (45.2)	68 (45.0)	21 (53.8)
Carvedilol, n (%)	3 (1.9)	8 (5.3)	0 (0.0)
Celiprolol, n (%)	1 (0.6)	3 (2.0)	3 (7.7)
Talinolol, n (%)	7 (4.5)	5 (3.3)	1 (2.6)
Propanolol, n (%)	5 (3.2)	2 (1.3)	0 (0.0)
Betaxolol, n (%)	1 (0.6)	1 (0.7)	0 (0.0)
Nebivolol, n (%)	12 (7.6)	15 (9.9)	0 (0.0)
Sotalol, n (%)	4 (2.5)	0 (0.0)	2 (5.1)
Daily β-blocker equal dose (mg) [†] , mean (SD)	5.3 (3.6)	5.3 (3.7)	5.8 (3.3)

[†] Calculations of daily β-blocker equal dose for bisoprolol based on the daily defined-dose concept using the ATC code [101]. No statistical differences between genotype groups (χ^2 -test or one-way ANOVA test, as appropriate)

ANOVA: Analysis of variance; SD: Standard deviation.

Table 3

Baseline characteristics of subjects with exercise data (study population) according to β -blocker use and *GNB3* C825T genotype.

Characteristic	No β -blocker				β -blocker			
	CC (n = 600)	TC (n = 543)	TT (n = 124)	p-value for trend [†]	CC (n = 157)	TC (n = 151)	TT (n = 39)	p-value for trend
Age, mean (SD)	49.2 (13.1)	50.6 (13.1)	49.2 (13.4)	0.165	60.6 (11.8)	59.5 (10.5)	61.0 (11.2)	0.612
Women, n (%)	302 (50.3)	284 (52.3)	62 (50.0)	0.773	79 (50.3)	80 (53.0)	21 (53.8)	0.866
Diabetes mellitus, n (%)	21 (3.5)	27 (5.0)	2 (1.6)	0.165	22 (14.0)	22 (14.6)	10 (26.3)	0.155
Hypertension, n (%)	208 (34.7)	188 (34.6)	42 (33.9)	0.985	128 (81.5)	130 (86.1)	32 (82.1)	0.537
Myocardial infarction, n (%)	4 (0.7)	6 (1.1)	1 (0.8)	0.725	15 (9.6)	11 (7.3)	5 (12.8)	0.521
Stroke, n (%)	5 (0.8)	4 (0.7)	3 (2.4)	0.201	4 (2.5)	5 (3.3)	3 (7.7)	0.287
Asthma bronchiale, n (%)	12 (2.2)	18 (3.3)	0 (0.0)	0.065	2 (1.3)	4 (2.6)	0 (0.0)	0.461
COPD, n (%)	20 (3.3)	27 (5.0)	3 (2.4)	0.238	9 (5.7)	8 (5.3)	1 (2.7)	0.756
Smoking status	0.503							
– Never smoker, n (%)	258 (43.0)	231 (42.5)	47 (37.9)		72 (45.9)	77 (51.0)	19 (50.0)	
– Ex-smoker, n (%)	184 (30.7)	151 (27.8)	42 (33.9)		61 (38.9)	59 (39.1)	16 (42.1)	
– Current smoker, n (%)	158 (26.3)	161 (29.7)	35 (28.2)		24 (15.3)	15 (9.9)	3 (7.9)	
Physically active, n (%)	283 (47.2)	247 (45.5)	59 (47.6)	0.823	56 (35.7)	65 (43.0)	18 (47.4)	0.264
BMI (kg/m ²), mean (SD)	28.1 (4.1)	27.9 (4.5)	27.9 (4.1)	0.780	28.3 (4.8)	27.9 (4.8)	27.7 (4.7)	0.605
Fractional shortening (%), mean (SD)	37.7 (7.7)	37.6 (7.4)	37.1 (7.3)	0.780	38.3 (7.6)	38.7 (8.4)	37.1 (7.4)	0.616
Medication								
Calcium-channel blocker	0.412							
– Diltiazem or verapamil, n (%)	7 (1.2)	3 (0.6)	0 (0)		0 (0)	1 (0.7)	0 (0)	

Characteristic	No β -blocker				β -blocker			
	CC (n = 600)	TC (n = 543)	TT (n = 124)	p-value for trend [†]	CC (n = 157)	TC (n = 151)	TT (n = 39)	p-value for trend
- Dihydropyridine, n (%)	21 (3.5)	22 (4.1)	7 (5.6)		20 (12.7)	22 (14.6)	3 (7.7)	
Digitalis, n (%)	2 (0.4)	4 (0.7)	1 (0.8)	0.494	6 (3.8)	3 (2.0)	1 (2.6)	0.624
Amiodarone, n (%)	0 (0)	0 (0)	0 (0)	1.000	1 (0.6)	0 (0)	0 (0)	0.545
ACE inhibitor, n (%)	54 (9.0)	52 (9.6)	13 (10.5)	0.859	59 (37.6)	60 (39.7)	14 (35.9)	0.878
Angiotensin receptor blocker, n (%)	14 (2.3)	24 (4.4)	2 (1.6)	0.077	22 (14.0)	19 (12.6)	8 (20.5)	0.477
Any antiobstructive drug	24 (4.0)	24 (4.4)	4 (3.2)	0.820	10 (6.4)	5 (3.3)	2 (5.3)	0.460
- β -adrenoceptor agonist	9 (1.5)	16 (2.9)	0 (0.0)	0.054	3 (1.9)	1 (0.7)	0 (0.0)	0.461
- β_2 -adrenoceptor agonist	0 (0.0)	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	0 (0.0)	1.000
- α -adrenoceptor antagonist	0 (0.0)	0 (0.0)	0 (0.0)	1.000	3 (1.9)	1 (0.7)	0 (0.0)	0.461
Exercise-related parameters								
Exercise duration (min), mean (SD)	9.6 (3.1)	9.2 (3.1)	9.6 (2.8)	0.058	7.9 (2.6)	7.9 (2.6)	7.6 (3.0)	0.823
Maximal power load (W), mean (SD)	162 (50)	155 (51)	162 (46)	0.052	134 (43)	134 (43)	131 (48)	0.887
Percent of age-predicted workload, mean (SD)	109 (27)	110 (30)	111 (27)	0.742	108 (30)	104 (31)	109 (34)	0.505
Resting HR (bpm), mean (SD)	81.2 (13.2)	80.2 (12.8)	80.2 (11.6)	0.415	71.2 (12.0)	69.7 (12.7)	66.3 (8.9)	0.072
Peak HR (bpm), mean (SD)	157.3 (19.3)	155.4 (19.7)	157.4 (18.8)	0.238	129.4 (23.4)	126.1 (20.9)	121.2 (22.0)	0.100
Percent of age-predicted HR [‡] , mean (SD)	92 (9)	92 (9)	92 (8)	0.817	81 (13)	79 (12)	76 (12)	0.074
Percent of age-predicted HR [‡] , mean (SD)	91 (9)	91 (10)	91 (9)	0.553	79 (13)	76 (11)	74 (12)	0.058
Chronotropic index, mean (SD) [‡]	0.85 (0.18)	0.85 (0.19)	0.85 (0.16)	0.853	0.66 (0.22)	0.62 (0.19)	0.59 (0.19)	0.075
Chronotropic index, mean (SD) [§]	0.84 (0.18)	0.82 (0.18)	0.84 (0.16)	0.580	0.63 (0.22)	0.59 (0.19)	0.56 (0.19)	0.108

Characteristic	No β -blocker				β -blocker			
	CC (n = 600)	TC (n = 543)	TT (n = 124)	p-value for trend [†]	CC (n = 157)	TC (n = 151)	TT (n = 39)	p-value for trend
Systolic resting BP (mmHg), mean (SD)	120 (16)	121 (16)	120 (5)	0.749	123 (18)	126 (17)	118 (14)	0.017
Diastolic resting BP (mmHg), mean (SD)	85 (11)	85 (10)	86 (11)	0.686	85 (12)	87 (12)	84 (12)	0.136
Systolic peak BP (mmHg), mean (SD)	199 (29)	196 (27)	200 (28)	0.147	192 (28)	191 (29)	188 (26)	0.693
Diastolic peak BP (mmHg), mean (SD)	92 (14)	91 (13)	91 (14)	0.665	89 (15)	90 (15)	92 (17)	0.582

Given values are unadjusted.

[†] Age-predicted HR calculated by the formula: $205.8 - 0.685 \times \text{age}$ [34]. (Separate analyses for subjects with β -blockers and without β -blockers.)

[‡] Comparisons between GNB3 C825T genotypes were made separately for subjects with β -blockers and without β -blockers, respectively, by χ^2 -test (nominal data) or one-way ANOVA test with Bonferroni's post-hoc test (continuous data).

[§] Age-predicted HR calculated by the formula: $220 - \text{age}$ [30].

ACE: Angiotensin-converting enzyme; ANOVA: Analysis of variance; BP: Blood pressure; bpm: Beats per min; COPD: Chronic obstructive pulmonary disease; HR: Heart rate; SD: Standard deviation; W: Watt.